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VOLUME 7 AUGUST, 1932 NUMBER 2

ON THE TESTING OF FIBRE-BOARD INSULATION[1]

BY C. D. NIVEN[2]

Abstract

A description is given in detail of the hot-plate apparatus which has been installed at the National Research Laboratories at Ottawa, primarily with a view to ascertaining the cause of the inconsistency in results obtained by different investigators on insulating wall board. Fibre boards composed either of bagasse or wood fibre were tested, and a migration of moisture from the hot to the cold side was found to occur. The conductivity of a board with a fairly high moisture content was found to decrease enormously during a long period of testing. The work indicates that the conductivity-density relation is essentially linear, but should be represented as a band rather than a line to allow for the effect of the structure of the board on the conductivity.

Introduction

The determination of the heat conductivity of fibre board has become a problem of considerable interest in connection with the heat insulation of houses. The apparatus used for this determination is known as the hot-plate apparatus and consists essentially of an electrically heated plate and two cold plates cooled by a circulation of tap water or brine. This type of testing apparatus has been accepted in the United States as the standard apparatus for measuring the heat conductivity of building boards, and a design described by Van Dusen (6) is used by the Bureau of Standards at Washington.

It is to some extent unfortunate that, when the hot plate was accepted as the standard apparatus, the design of the apparatus and the conditions of test were not specified in detail. The consequence of this lack of specification has been confusion in the results: almost every conceivable suggestion has been made to explain the discrepancies, even to the extent of questioning the truth of Fourier's law. It was mainly with a view to discovering where the difficulty lay in making these tests that the work described in this communication was undertaken.

The following suggested themselves as reasonable explanations for the confusion in the results: first, the apparatus used by some of the experimenters was of unsuitable design and therefore gave erroneous results; second, there actually were variations in the samples of materials used for the tests, although

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Contribution from the National Research Laboratories, Ottawa, Canada.
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these samples were ostensibly the same in so far as trade name was concerned; third, the experimental procedure varied in the different tests; fourth, the moisture content varied in the different samples tested; and fifth, in the period of the tests, thermal equilibrium had not been reached. The recent paper by R. Ruedy (5) shows that the last possibility can be ruled out as it should theoretically take less than two hours to come within 0.1% of the final value.

Apparatus

General

Before deciding on the design of the hot plate to be installed at the National Research Laboratories at Ottawa, various types of apparatus described in the literature were considered. The design of Van Dusen appeared to be both simple and accurate, and was therefore taken as the model. A complete description of this apparatus has been given by Van Dusen (6) and need not be repeated here. The following slight changes were made: the overall dimensions were increased from 12 by 12 in. to 18 by 18 in.; the width of the guard ring on the outside of the hot plate, *i.e.*, the copper guard plate, was increased from 2 to 3 in.; and the width of the bakelite, on which the guard-ring element was wound, was increased from ½ to 1 in.

These modifications necessitated changes in the spacing of the heating element: thus, along one edge the spacing was 1 in. but along the edge at right angles the spacing was 1.029 in. This figure was determined by dividing 18 in. into 17½ parts. The ribbon used in winding had a resistance of about 0.15 ohms per ft., giving a total resistance under the central square foot of the hot plate, *i.e.*, under the effective area, of slightly more than five ohms. The thickness of the copper plates used on the outside of the hot plate, *A*, was ⅛ in., and the thickness of the bakelite on which the constantan ribbon was wound was $\frac{3}{16}$ in. The total thickness of the hot plate was thus about $\frac{7}{16}$ in. (See Fig. 1).

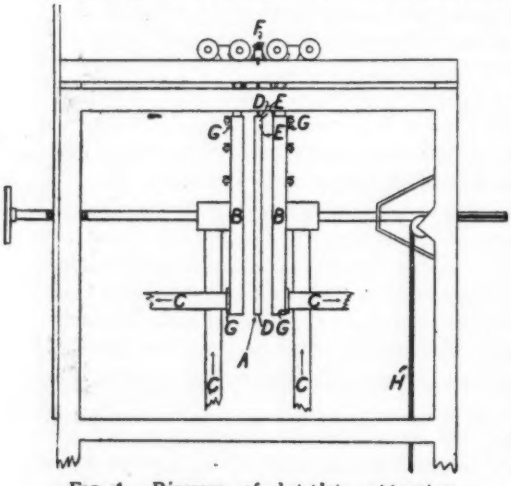

FIG. 1. *Diagram of hot-plate apparatus: A, hot plate; B, cold plates; D, current leads to main heating coil; E, current leads to guard-ring heating coil; F, nine thermocouple-lead wires from hot and cold plates; G, studs used in thickness measurements; H, cable suspending weight; C, brine pipes.*

The cold plate consisted essentially of an aluminium casting about 1 in. thick. In this was cast a channel 2 in. wide and ½ in. deep, following a labyrinthine course from the centre to the edge. A plate of copper ⅛ in. thick covered the channel and was held tightly against the aluminium casting by means of countersunk brass screws in the

web between the turns of the channel. The copper face of the completed cold plate was placed next to the sample. A little lacquer was found to be effective in preventing leakage of the brine past the screwheads. To avoid the trapping of gas at the top of each turn of the channel, pet cocks were screwed into the casting to provide escapes.

In order to press the plates and samples together during the test with the same pressure each time, two push bars were arranged at opposite sides of the apparatus to press against the middle of each of the cold plates. One push bar was screwed through the frame of the apparatus, while the other was pulled inwards by two cables passing over pulleys and carrying a heavy weight. This weight exerted a pressure on the plate of 35 lb. per sq. ft.

To heat the main element of the hot plate, current was supplied from a group of five six-volt batteries. These batteries had to be carefully charged, and it was found necessary to have a spare set; the one set could then be charging while the other was discharging. The guard-ring element was heated by direct current from a generator, while the cold plates were cooled by brine pumped through them from a tank connected to a refrigeration machine.

Calibration

The measurements of heat conductivity on a hot-plate apparatus involve essentially three quantities, the heat energy supplied per hr. per sq. ft. of surface, the temperature difference between the hot and cold surfaces which this energy supply can maintain, and the thickness of the insulator separating the two surfaces. The first of these three—namely, the energy supplied per hour—involves not only the measurement of the current but also of the resistance per square foot under the effective heat transmitting surface. It is in order to eliminate the complication of a resistance varying with temperature that the constantan ribbon is used in this type of hot-plate apparatus.

In order to measure accurately the resistance under the effective surface, the following procedure was adopted. The hot plate was put together without inserting the micanite insulation between the copper plates and the heating element. The centres of the small portions of ribbon, exposed to view in the saw cut separating the central and guard ring portions of the copper plate, were marked by drawing a pencil round the saw cut. The plate was then taken apart and the resistance of the pieces of ribbon between the pencil marks was measured. The potentiometer method was employed, so that it might be possible to use point contacts, pressing on the pencil marks, for the potentiometer-lead contacts.

The values of the resistance measurements thus obtained were then added together in order to ascertain the total resistance under the effective heating surface. This figure was checked against the fraction of the resistance of the whole heating element, obtained by measuring its resistance and multiplying by the ratio of the area under the central portion of the copper plate to the total area of the bakelite plate, taking care to allow for the edges of the bakelite plate, and the width of the saw cut. An additional check was made by measuring the lengths between the pencil marks and multiplying by the specific resistance of the ribbon.

When the total resistance had been accurately ascertained, the resistance per sq. ft. was calculated by dividing by the area enclosed by the pencil mark in the saw cut. When this figure is multiplied by the constant 3.4145 and by the square of the current measured in amperes, it represents the number of B.T.U.'s per hr. supplied per sq. ft. of surface area. The accuracy of measurement of the thermal energy supply is also dependent on the accuracy of current measurement. The measurement of the latter is by no means simple on account of the difficulty in keeping the current constant. It is necessary to watch the current reading on an ammeter during the test, making frequent adjustments of the rheostat in series with the heating element in order to maintain the current at a constant value. With the equipment used by the writer errors of about 0.3% were possible so that, due to errors in current measurement alone, an error of 0.6% might be introduced in the final expression for conductivity owing to the fact that current appears as the second power in this expression.

The second important quantity to measure is the temperature difference. This was measured by means of a copper-constantan thermocouple, *F*, one of the two junctions being fixed to the hot plate and the other to one of the cold plates. The thermocouple e.m.f. was measured on a Leeds and Northrup type K potentiometer. Readings could be taken with an accuracy of about 0.1°F. on a temperature difference of 60°F. The possible error, therefore, which might arise from faulty measurement of temperature difference appears to be small. The real accuracy, nevertheless, depends not only on the accuracy of the original calibration of the thermocouples, but also on the freedom of the apparatus from parasitic e.m.f.'s. The thermocouples used were accurately calibrated over the range in which the tests were to be made and the points were plotted on the same graph as the calibration values of L. A. Adams (1). The writer's calibration was within 0.25% of that of Adams.

The freedom from parasitic e.m.f.'s depends on the efficiency of the micanite insulation between the heating element and the copper plate to which one junction of the thermocouple is attached. If the electrical leak is to be negligible when the 110-volt line is connected to the heating circuit, this insulation must be efficient. In the apparatus described above, the resistance between the plate and the heating element measured more than 10 megohms. After the final readings on the thermocouples have been taken in an actual test, it has been found to be a good plan to cut off all the electrical circuits and ascertain if there is any change in the thermocouple reading. If there is none the micanite insulation is satisfactory.

It is very important to maintain the temperature of the cold plates at a constant known value, in order that the thermocouple reading may be converted to temperature difference, since the e.m.f.-temperature curve is not strictly linear.

The third essential measurement in making a test is the thickness of the sample. To measure this thickness the method employed by the Bureau of Standards was used. Into each of the cold plates, at the corners, four studs were screwed permanently. After cleaning any rust off the heads of these

studs and dusting the faces of the plates themselves, the push bars were allowed to draw the apparatus together. The distances between the four pairs of studs were then measured on vernier calipers. The sheets were then put in between the plates and the distances again measured. Half the mean of the four differences between these two sets of measurements gave the effective thickness of a sheet. In measuring soft materials the push bar should be clamped so that the constant pressure during test will not change the thickness of the sample. It is rather difficult to estimate the error in the measurement of thickness as it is possible to repeat a measurement on one-inch material to 1/1000, yet in many cases, for no apparent reason, measurements have not repeated to even 5/1000. It does not seem justifiable to conclude that the calipered measurement is at fault when the figures do not repeat. Obviously the position and orientation of the sheets with regard to the apparatus might have changed and so caused a change in the measured thickness. This would be accompanied by a change in the heat insulation due to the small additional air space between the sheets and the plates, which, as Van Dusen points out, may be considered for all practical purposes as having the same conductivity as a wall board. This would result in a change in the total heat resistance without necessarily altering the specific heat resistance of the sample to any appreciable extent. In short, when a measurement of thickness on the same board containing the same amount of moisture will not repeat, there may be a corresponding change in the energy-supply measurement, which to a large extent cancels what might otherwise be considered as an error.

Operation

The accuracy of the three measurements discussed above, namely, energy supply, temperature difference, and thickness, determines the calibration of the apparatus, but however carefully these measurements are made, the results which the apparatus yields will not be accurate unless the experimenter makes certain that temperature equilibrium has been reached when the final readings are taken, and that, for some time before the equilibrium readings are taken, there has been no lateral loss of heat from the effective portion of the hot plate.

It is in order to prevent any lateral flow of heat that the apparatus is equipped with a guard ring. Van Dusen (6) explains the method of determining when the temperature of the guard ring is the same as that of the main portion of the plate. The method consists of adjustment of the guard-ring current until there is a zero reading on a number of thermocouples in parallel, the junctions of which are on the guard ring and on the main portion of the hot plate. It is as essential to watch this thermocouple reading closely as it is to maintain a constant current through the main heating element.

Mainly on account of the difficulty connected with maintaining electrical currents absolutely constant over long periods the following method was used to obtain the final readings. The current was set and maintained at a particular value after the guard-ring current had been adjusted to maintain a zero value on the guard-ring thermocouples. If the temperature difference between the

hot and cold plates increased, the main current was cut down and if the temperature difference decreased the main current was increased. In this way the current could be adjusted around a value above which the temperature difference increased and below which the temperature difference decreased. By narrowing down the limits the equilibrium current for a particular temperature difference was obtained. Obviously, when the limits had been narrowed down the importance of maintaining a zero reading on the guard ring thermocouple was increased. This method of obtaining the equilibrium readings cannot of course be applied until the equilibrium temperature gradient has been nearly established, and therefore overheating the hot plate at the start speeds up the test.

The Effect of Moisture in Samples under Test

In order to compare the results of observations on boards of different densities conductivity was plotted against density. The results for the two kinds of wood-fibre board and for bagasse-fibre board all lay on a more or less smooth curve, but there were features about these results which were not altogether satisfactory. On a two-inch board it was found that after 48 hr. of testing the value for the conductivity was still changing and the question at once arose as to whether six hours had been a long enough period of test for the one-inch sheets. Experiments lasting about 24 hr. were carried out to check the results of the six-hour tests, and it was found that while the results on a cork board sample, measured after 6 hr., could be almost repeated after 24 hr., the results on ordinary fibre boards changed considerably according to the duration of the test.

During the summer it had been noticed that the fibre boards were gaining in weight while the weight of the cork board remained approximately constant. This suggested that the moisture content was in some way connected with the change in conductivity after long periods of testing, pointing to the necessity of making a systematic investigation of the variation in conductivity of fibre boards containing different amounts of moisture. A humidifying chamber was constructed so that boards would be in a moist atmosphere without being in contact with water and the following series of experiments was then conducted on the fibre boards.

The board was first taken from a collection which had been lying in the atmosphere of the room, and was subjected to the heat-conductivity test; equilibrium readings were taken after about 6 hr. and again after about 24 hr. The board was then taken out of the testing apparatus and dried, the conductivity test repeated and the board was then put into the humidifying chamber for several days. After there had been a considerable gain in weight the conductivity test was repeated. Each time, the boards were weighed before and after testing. During a test the weights of boards with high moisture contents decreased and the weights of very dry boards increased. The conductivities after 6 and 24 hr. were plotted against the weight before testing and diagrams, of which Fig. 2 is typical, were obtained. Clearly, the duration of test was all-important, and from the fact that a board which was initially

dry gained in weight while a board which was initially high in moisture lost in weight, the suggestion was that the moisture in the board migrated in such a way as finally to set up a moisture gradient. One test was particularly interesting; a wood-fibre board, initially containing as much as 13.1% of moisture, was tested, and the equilibrium readings were taken after 6, 24, 48 and 72 hr. in the hot-plate apparatus. The conductivities were 0.380, 0.367, 0.354 and 0.349 respectively. When the board was taken out of the apparatus there was a thin sheet of ice on the surface next the cold plate; yet in spite of this fact, the weight of the board had decreased during the test.

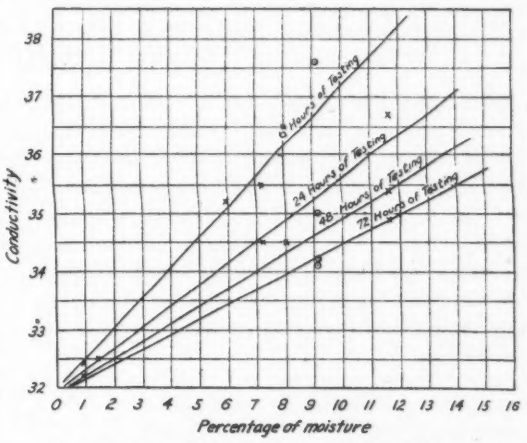

FIG. 2. *Relation between conductivity and percentage of moisture in boards under test: ×, temperature 43° to 53°F.; ⊙ temperature 67° to 71°F. The ordinates of the points of intersection of radial lines with vertical lines indicate conductivities after various times.*

There was no further doubt as to what was happening. Moisture was migrating from the warm side of the board to the cold side and the effect was virtually the same as a gradual drying of the warm side. The sheets which were dried in the oven were dried at only 150°F. and may have retained a little moisture, thus accounting for the slight variation with length of test, even on the dried sheets.

When these diagrams were obtained, the necessity of reviewing the whole subject became more apparent than ever. The presence of moisture was not only affecting the conductivity of a sample by changing, so to speak, its physical properties generally, but was also bringing in a time factor due to the gradual migration of moisture from the hot to the cold side. The question then arose as to whether the 6-hr. readings or the 24-hr. readings gave the true value of thermal conductivity. The 24-hr. readings were probably the more reliable, yet the 6-hr. readings gave a more real measure of the conductivity of a board with a particular moisture content. This leads to the conclusion that the hot plate is not the ideal apparatus; in actual use a wall board must be continually changing its moisture content, depending on the humidity of the room. As, however, the hot plate is the only apparatus so far developed which has proved even tolerably satisfactory, the only thing possible at present is to tabulate the results giving details of the various factors involved in each particular case.

The Effect of Mean Temperature

The conductivity of a fibre board increases as the mean temperature during test is raised. To express the relation between mean temperature and conductivity, Van Dusen and Finck (7) used the equation

$$K_t = K_{90}[1+\alpha(t-90)],$$

where K_t is the conductivity at temperature t°F. and K_{90} at 90°F., t is the mean temperature of the sample under test, measured in °F., and α is a constant referring to the particular material in question. In order to obtain data which will determine α it is necessary to have two mean temperatures sufficiently far apart that the experimental error may not mask the small change in conductivity with temperature. It was necessary, therefore, to use a very much larger temperature difference in obtaining the reading at higher temperatures than that which had been used in the rest of the work, because there was no adequate equipment on hand to raise the temperature of the cold plate sufficiently.

TABLE I

VALUES OF α FOR THE EQUATION OF FINCK AND VAN DUSEN (7) AS DETERMINED BY THE AUTHOR AND BY FINCK AND VAN DUSEN

Material	Value of α as determined by:	
	Finck and Van Dusen	Author
Bagasse	0.0013	0.00096
Bagasse	0.0012	.....
Bagasse	0.0012	.....
Wood fibre No. 1	.....	0.0013
Wood fibre, low density	.....	0.0012
Wood fibre No. 2	.....	0.0010
Balsam wool	0.0009	
Fibrofelt	0.0011	.....
Insulite	0.0020	.....

The values of α found by the writer and shown in Table I are in satisfactory agreement with the values given by Van Dusen and Finck (7) and clearly indicate that a variation of 10°F. in mean temperature does not make any significant difference in the results, at least, not unless the experimental error can be reduced below 2%. There was, however, a question as to whether a test carried out with a cold-plate temperature below 32°F. would give results differing from a test with a cold-plate temperature well above 32°F. This was especially important with regard to the migration of moisture, as it was suggested that perhaps a low cold-plate temperature favored this phenomenon. A test was made on a specially humidified board, and the same gradual decrease in conductivity was observed during a 72-hr. test that had been observed during a similar test with a cold-plate temperature below 32°F. The results of this test are shown in Fig. 2. It may be concluded therefore that the migration of moisture is primarily dependent not on the temperature of the cold plate, but on the temperature difference maintained between the hot side and the cold side.

Tabulation of Results

The following have been tabulated in Tables II and III: material, thickness, density, moisture content, duration of test, mean temperature, temperature difference and conductivity. The temperature and humidity of the room

during test are not of great importance, except in so far as they may under exceptional conditions upset a test; for instance, certain very hot days inter-

TABLE II

DATA FROM HEAT-CONDUCTIVITY EXPERIMENTS WITH VARIOUS INSULATING BOARDS, COLD-PLATE TEMPERATURE BELOW 32°F.

Thickness, in.	Moisture %	Density, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity
*Wood fibre, No. 1, ½ in.**										
0.540	6.7	18.2	6	46	55	0.395	..	..	..	.....
0.532	6.7	18.2	6	44	54	0.390	..	..	..	.....
0.532	6.7	18.4	5	46	57	0.381	24	47	56	0.377
0.518	0.0	17.6	6	45	57	0.357	24	46	57	0.357
*Wood fibre, No. 1, 1 in.**										
0.815	6.8	18.5	5	49	56	0.405	..	..	..	.....
0.816	7.4	18.8	5	46	57	0.406	23	47	56	0.400
0.797	0.8	18.0	6	45	56	0.377	25	45	55	0.370
*Wood fibre, No. 1, low density, 1 in.**										
0.993	7.3	10.6	6	45	57	0.344	29	45	59	0.331
0.979	0.8	10.0	6	44	56	0.325	24	48	57	0.320
0.989	6.7	10.6	5	42	57	0.347	24	42	57	0.336
0.979	0.4	9.98	6	45	56	0.323	27	44	56	0.318
*Wood fibre, No. 2, ½ in.**										
0.431	3.8	16.6	6	45	58	0.369	..	..	..	.....
0.525	3.8	16.7	6	45	54	0.369	..	..	..	.....
0.526	?	15.1	6	43	55	0.362	..	..	..	.....
0.563	4.8	15.1	6	44	55	0.362	..	..	..	.....
0.530	5.6	15.4	6	44	54	0.362	..	..	..	.....
0.475	3.9	17.1	6	44	53	0.362	..	..	..	.....
0.538	7.5	13.7	5	45	53	0.347	27	43	51	0.342
0.530	5.4	16.2	5	48	55	0.366	25	46	53	0.358
0.529	7.2	15.8	5	45	54	0.360	26	43	50	0.362
0.516	0.0	15.0	5	46	55	0.339	24	44	52	0.339
0.535	10.2	16.1	6	46	55	0.378	26	44	52	0.367
0.521	5.6	17.1	6	43	56	0.364	25	48	55	0.359
0.530	7.9	17.1	..	..	..	..	30	50	53	0.375
*Wood fibre, No. 2, 1 in.**										
1.115	5.9	16.0	6	43	54	0.352	..	..	..	.....
1.116	8.0	13.4	6	46	51	0.365	29	48	53	0.345
1.092	1.4	12.7	....	..	..	..	26	49	57	0.325
1.089	0.9	12.7	5	45	54	0.324	29	44	54	0.322
1.132	11.6	13.7	5	48	55	0.380	30	47	56	0.367
1.116	7.2	13.2	..	..	..	..	34	53	55	0.345

**This is nominal thickness, the exact thickness is shown in the column headed, "Thickness".*

TABLE II—*continued*

Thickness, in.	Moisture %	Density, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity
Wood fibre, No. 2, loose										
1.099	5.1	5.0	5	44	56	0.311	24	43	54	0.304
1.075	5.1	3.1	7	45	56	0.290	27	44	57	0.284
1.095	5.1	7.3	..	..	..	..	23	45	56	0.298
1.092	5.1	7.3	5	44	56	0.307	25	47	55	0.298
*Bagasse, ½ in.**										
0.472	5.8	15.5	6	43	54	0.361	..	..	..	....
0.471	5.8	15.3	6	42	54	0.362	..	..	..	....
0.470	6.4	15.5	5	45	56	0.353	28	47	56	0.348
0.456	0.0	14.9	6	44	56	0.334	25	46	56	0.333
*Bagasse, 1 in.**										
0.897	6.6	18.2	6	48	55	0.403	..	..	..	....
0.879	1.4	17.8	6	47	55	0.373	29	48	56	0.363
0.920	9.6	18.3	6	45	56	0.396	29	48	55	0.381
0.895	7.4	18.4	....	..	..	....	29	53	59	0.375
0.881	1.3	17.8	....	..	..	....	29	49	53	0.361

**This is nominal thickness, the exact thickness is shown in the column headed, "Thickness".*

fered with the cooling; or, again, accumulation of moisture on the edges of the plates and samples on a humid day might possibly interfere with the thermocouples. This was largely obviated by the use of pads placed around the edges of the plates so that the moist air was kept away from the material under test. To a large extent the moisture content of a sample was dependent upon the particular date of testing and the humidity at that time. The temperatures of the hot and cold plates can be calculated from the temperature difference and mean temperature data. The items which have been tabulated are too numerous to permit of expressing the results in graphic form without making certain compromises. Conductivity has been taken as the dependent variable, and density, which is the most important of the other items, has been taken as the independent variable. Moisture content and mean temperature have been taken as parameters, and thickness and particular type of material have been ignored. The influence of these factors will be discussed briefly below; only the results on tests conducted during the day and continued overnight have been plotted.

The three lines on Fig. 3 relate to tests at different temperatures on dried samples, while Figs. 4 and 5 relate to samples containing moisture. Obviously the points do not lie strictly on the straight lines even when the data have been

TABLE III

DATA FROM HEAT-CONDUCTIVITY EXPERIMENTS WITH VARIOUS INSULATING BOARDS, COLD-PLATE TEMPERATURE ABOVE 32°F.

Thickness, in.	Moisture %	Density, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal conductivity
*Wood fibre, No. 1, low density, 1 in.**										
0.975	0.5	10.0	5	62	56	0.327	25	63	55	0.330
0.980	3.8	10.4	6	67	56	0.342	30	67	56	0.327
0.974	0.6	10.0	6	67	55	0.330	25	67	56	0.325
0.990	7.5	10.6	5	67	56	0.361	24	67	55	0.341
0.970	0.0	10.0	5	72	56	0.321	25	70	53	0.327
0.970	0.1	10.0	6	98	108	0.342	30	98	111	0.346
*Wood fibre, No. 1, 1 in.**										
0.797	0.8	18.0	6	68	56	0.382	24	65	55	0.377
0.793	1.1	18.1	5	70	56	0.377	25	69	54	0.371
0.793	0.0	17.9	5	97	101	0.394	24	103	89	0.399
*Bagasse, 1 in.**										
0.898	5.2	17.9	6	67	56	0.373	25	66	54	0.371
0.879	0.3	17.5	6	66	54	0.360	25	66	54	0.361
0.875	0.2	17.5	6	71	54	0.362	25	71	54	0.363
0.875	0.0	17.5	5	92	96	0.374	25	93	99	0.377
*Bagasse, ½ in.**										
0.467	4.7	15.3	5	66	51	0.347	24	64	50	0.348
*Wood fibre, No. 2, 1 in.**										
1.117	9.1	13.6	5	68	55	0.376	24	71	59	0.350
1.083	0.3	12.7	6	98	104	0.353	30	110	107	0.344
1.081	0.0	12.7	6	69	54	0.329	25	68	54	0.328

**This is nominal thickness, the exact thicknesses are shown in the column headed "Thickness".*

carefully classified in this way. It is also apparent that the materials of the highest and lowest densities have conductivities considerably greater than one would expect if there existed a linear relation between conductivity and density. At first sight one might be tempted to draw a curve through these points and conclude that at high density the conductivity rose rapidly and that at low density it tended to approach a constant value. In a paper by Finck (4) curves are given showing the relation of density to thermal conductivity for wood pulp and bagasse. Both of these curves indicate that the relation is not linear, but on closer examination it is seen that the curve for wood pulp is constructed from points whose distances from points determining

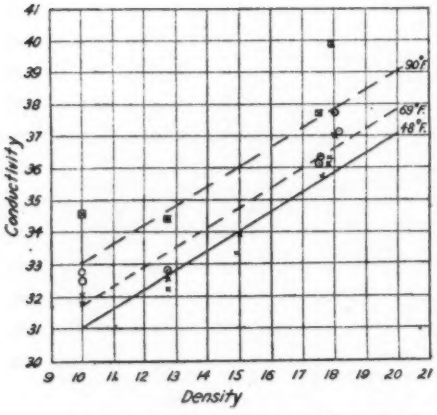

FIG. 3. *Relation between conductivity and density in lb. per cu. ft. of boards under test: ×, temperature 44° to 49° F.; ⊙, temperature 65° to 72° F.; ⊠, temperature 93° to 110° F. Samples dried in oven at 150° F.*

a linear relation are less than the experimental error. In the case of the bagasse curve, Finck's work indicates a very definite trend which would imply that bagasse of density 18 lb. per cu. ft. would have a conductivity of 0.45. This is hard to reconcile with work done on bagasse board.

Some data on wood-fibre board trimmings tested by the writer, when combined with the data on actual boards, point to the conclusion that the density-conductivity relation is essentially linear, and that the departures from the straight-line relation are due to packing or structure. Fig. 6 shows the data for fibre boards of various materials containing between 5 and 8% of moisture, plotted on the same diagram as some data on the trimmings of one of the wood-fibre boards. The trimmings were packed at different densities. This diagram seems to show that the linear relation holds good between the densities of 3 and 18 lb. per cu. ft. The departures from linearity amount to as much as 5 or 6%. It is interesting to note that those materials which have conductivities considerably higher than that which the linear relation implies are one-inch boards. The deviation from the linear is greater than any detectable difference between a bagasse and a wood-fibre board of the same density, and there is therefore considerable justification for plotting results for different fibre boards on the same graph.

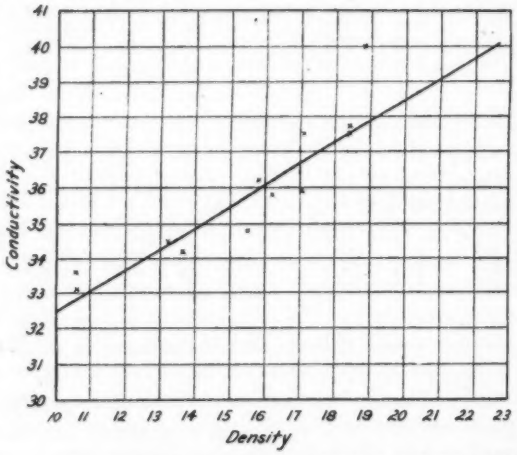

FIG. 4. *Relation between conductivity and density of boards under test: temperature, 42° to 53° F.; moisture, 6.7%.*

The fibres of bagasse raw material are longer than wood fibres and would therefore be more likely to lie in the plane of the board than perpendicular to it. In thick boards the tendency for the fibres to lie in the plane of the board might decrease, especially if the fibres were short. This probably

accounts for the fact that the half-inch wood-fibre board of density 17.6 has a lower conductivity than the one-inch bagasse board of density 17.8, while the latter again has a lower value than the one-inch wood-fibre board of density 18.0. The thickness, structure, and material of the board are so interlocked in their effect on conductivity that it would be unwise to differentiate between the insulating qualities of bagasse and wood fibre without carrying out a large number of tests on numerous boards taken at random from stock. Care would have to be taken in such a series of tests to see that the boards were of the same density and of the same nominal thickness; *i.e.*, a half-inch board should not be compared with a one-inch board planed down to half an inch. If the tests were not performed on bone-dry material it would be essential to see that the moisture contents of the materials under comparison were the same.

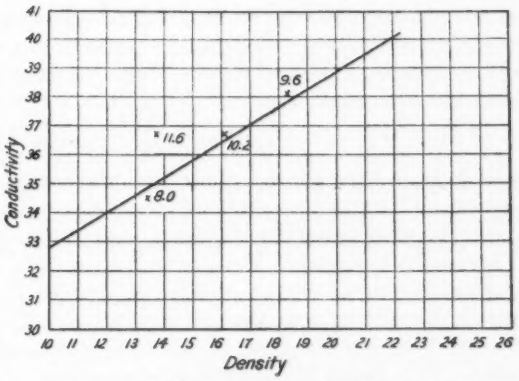

FIG. 5. *Relation between conductivity and density of boards under test: ×, temperature 44° to 48° F.; moisture about 9.4%. The number written in at the point indicates actual moisture content of sample.*

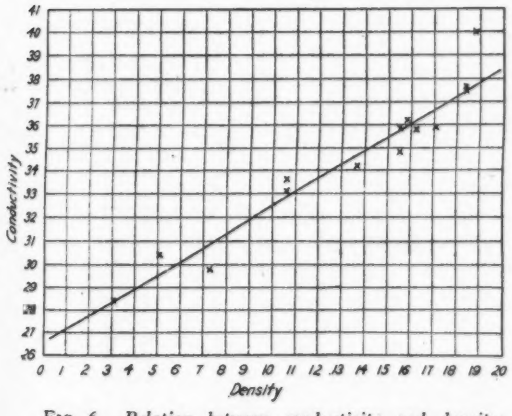

FIG. 6. *Relation between conductivity and density: temperature, 42° to 53° F.; moisture 5 to 8%.*

Before leaving the discussion on the effect of the packing of the particles of fibre in a wall board on its thermal conductivity, reference might be made to a paper by Awbery (3) in which it is concluded on theoretical grounds that the thermal conductivity of a body is determined, not by the size of its component particles, but by their shape, and by the way in which they are packed. Reference might also be made to Finck's paper (4) on the mechanism of heat flow in fibrous materials, in which it is shown that the conductivity is greater when the fibres are packed along the direction of heat flow than when they are packed at right angles. Finck's work explains why a thick board may have a higher conductivity than a thin board composed of the same material and packed at the same density, while Awbery's work explains why the compara-

tively large bagasse fibres, when compressed to form a board, give a resultant thermal conductivity differing very little from that of a board composed of very small wood fibres, provided of course that the density of the finished board is the same in either case.

Comparison with Other Results

In order to compare the results described above with those of other experimenters, Table IV has been constructed. Column 5 contains the values which the writer's work leads him to believe are representative of fibre boards of particular densities. They have been arrived at by examining the graphs on Figs. 3, 4 and 5, and by using the equation $K_t = K_{90}\,[1 + \alpha\,(t-90)]$. To convert a value for a sample containing 5 to 8% of moisture to the value for the dry sample, a reduction of 0.014 has been made on the heat conductivity value. This figure is obtained by subtracting the value of the conductivity for a particular density, as given in Fig. 3 by the line marked 48°F., from that given in Fig. 4 for the same density.

By applying these corrections for temperature and moisture, the results are in a form in which they can be compared with those of Finck. The agreement

TABLE IV

A COMPARISON OF HEAT-CONDUCTIVITY VALUES OBTAINED BY THE AUTHOR, AND BY OTHER WORKERS

Density of material tested	Conductivity as determined by			Author's representative value
	Finck	Finck and Van Dusen	A. S. H. V. E.	
Bagasse				
4.0	0.277	—	—	0.290
4.8	0.285	—	—	0.295
6.7	0.296	—	—	0.307
7.4	0.315	—	—	0.311
8.9	0.337	—	—	0.320
12.1	0.375	—	—	0.343
12.2	—	0.325	—	0.344
13.2	—	—	0.340	0.350
13.5	—	—	0.337	0.351
14.2	0.403	0.358	—	0.356
14.8	—	0.342	—	0.359
Wood pulp				
3.0	0.304	—	—	0.283
4.9	0.299	—	—	0.295
6.6	0.309	—	—	0.306
9.0	0.318	—	—	0.320
Insulite				
16.2	—	0.336	—	0.368
16.5	—	—	0.348	0.370
16.9	—	0.336	0.340	0.372

in the case of wood pulp is satisfactory, but at higher densities the agreement with bagasse is decidedly poor. However, when the results in the American Society of Heating and Ventilating Engineers' Guide Book (2) as well as the results in a paper by Finck and Van Dusen (7) are taken into consideration, it is found that the writer's mean values are between the values given in these two references and Finck's values as given in the first column in Table IV —provided of course due regard be paid to density. Moisture content has been assumed to be zero in these results, and where necessary the conductivity has been corrected to apply to a test carried out at a mean temperature of 90°F.

Conclusions

Apparatus

1. In view of the fact that moisture can migrate during a test from the hot side of the sample to the cold side, the hot-plate is not an ideal apparatus for testing the thermal conductivity of fibre wall boards in the commercial or undried state.

2. If wall boards are dried before testing, fairly consistent results can be obtained on the hot-plate apparatus. By improving the experimental equipment, advances in the accuracy of the test might be made, as for instance by the installation of an automatic control to maintain the current constant to 1/1000 of an ampere, and by arranging for a very accurate thermostatic control on the brine tank.

Data

1. It has been definitely proved that tests carried out on homogeneous fibre boards are of little value unless the moisture content is stated. Tests on samples containing moisture are inclined to be inaccurate even if the moisture content is stated, because there is a gradual migration of moisture from the hot side to the cold side. The result of this migration phenomenon is virtually the same as the effect of a very slow drying oven. Therefore, tests on dry sheets only should be considered as accurate. Tests on undried sheets have a certain value if both the moisture content and the duration of test be stated.

2. When the moisture contents of two fibre boards of the same thickness are equal, the really important factor in establishing their relative merits as insulators is density. It is unwise to compare the product of one manufacturer with that of another without taking into account the densities of the two products and ascertaining if these densities are typical of the manufacturer's product in that particular quality and nominal thickness of material. Results lead to the conclusion that the conductivity of fibre board between the densities of 3 and 18 lb. per cu. ft. is given by a linear band on a density-conductivity diagram.

3. Peculiarities in the structure of boards affect the thermal conductivity and therefore the points on the density-conductivity diagram do not lie accurately on a straight line but on a band. The differences between the conductivity values for different thicknesses and makes of wood-fibre board are as great as, or greater than, the differences between values for the wood-

and bagasse-fibre boards. When all factors, including density, are taken into account, bagasse and the two kinds of wood-fibre board tested by the writer did not differ much in insulating qualities.

4. Wood fibre in the loose state has been tested for thermal conductivity at a density as low as 3 lb. per cu. ft. and down to that density no evidence has been found to substantiate the belief that the thermal conductivity of fibre board increases with decrease of density.

5. A variation of 10°F. in the mean temperature of the sample appears to be of little importance in testing homogeneous fibre boards. Only with large changes of mean temperature does the change in thermal conductivity make its appearance. The magnitude of this change for fibre board has been determined and agrees fairly well with the work of other investigators.

Note.

Since this paper was written, a report has been found by the writer giving the results of some recent work by Cammerer on the effect of moisture content on the thermal conductivity of brick. This report appears on page 66 of "Heating and Ventilating", for April, 1932. Dr. Cammerer has found the influence of moisture content to be even more important in the determination of the thermal conductivity of brick than the writer has found it to be in the case of fibre board.

In the same issue of "Heating and Ventilating" there appears an article by Berestneff entitled "Moisture—its influence on the heat conductivity of building materials". Berestneff, after taking full account of Dr. Cammerer's work, remarks in summing up, "that all tables for coefficients of conductivity should also indicate the moisture content at which the values given in the tables are found." In the conclusion of the present communication the first sentence under "Data" expresses, as follows, almost exactly the same idea, "that tests carried out on homogeneous fibre boards are of little value unless the moisture content is stated."

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A NEW SWEEP-CIRCUIT DEVICE FOR THE CATHODE-RAY OSCILLOGRAPH[1]

By Geo. S. Field[2]

Abstract

On account of the frequency limitation of the ordinary sweep circuit used with the low-voltage cathode-ray oscillograph tube, a new circuit was developed for use in the analysis of higher-frequency alternating voltages and currents. This circuit has been employed to generate saw-tooth waves at frequencies up to 50,000 cycles per sec., and can probably be made to work at higher frequencies if necessary.

Introduction

The circuit (1) commonly employed for a time-base in connection with cathode-ray oscillography involves a discharge tube such as a neon glow tube or mercury-vapor thyratron. In such a circuit, a battery, high resistance and condenser are connected in series, while the discharge tube is connected in parallel with the condenser. Voltage gradually builds up across the condenser, until it reaches the break-down value for the tube. The condenser immediately discharges, the tube ceases to glow, and the process repeats itself. The result is a saw-tooth wave of voltage across the condenser, and it is this voltage which is connected to the oscillograph deflection plates.

This circuit works very well up to possibly 10,000 cycles per sec., but at higher frequencies difficulties arise on account of the time required for ionization and de-ionization of the gas in the discharge tube. The time-base described in this paper was developed to be independent of a gaseous discharge, and has not therefore the same frequency limitation.

The Circuit Used

In Fig. 1 the circuit is shown schematically. Tubes A, B, G and C are ordinary receiving valves, such as the UY-227. The diode D may be a 230 or 199 with the grid and plate connected together. Resistance r_1 has a value of 25,000 ohms; r_2 and r_3 are each of 50,000 ohms. V is 200 volts. The terminals T_1 are connected to the voltage whose wave-form is being analyzed, and the terminals T_2 go to the oscillograph. The action of the circuit is as follows.

During the positive half of a cycle of the voltage at T_1, the grid of A is positive and A will therefore pass electrons from filament to plate. Tube B will pass current if its grid voltage is not too greatly negative, and this voltage is so adjusted that when c_1 is discharged and no current is flowing through r_1, the grid has zero potential. Current accordingly flows through B, and causes a potential drop through r_1 as voltage builds up across c_1. With c_1 charging, the voltage across r_1 is amplified by G and C to produce a slightly positive potential on the grid of B, which causes more current to flow through B. Voltage V, or a large fraction of it, now appears across c_1.

[1] *Manuscript received June 2, 1932.*
Contribution from the National Research Laboratories, Ottawa. This paper was read at the meeting of the Royal Society of Canada, May 1932.
[2] *Junior Research Physicist, National Research Council, Ottawa.*

This condenser then begins to discharge through D, and the potential drop across r_1 is reversed. This drop is amplified as before to produce a high *negative* potential on the grid of B. Hence no current flows through B until c_1 has discharged. When this has occurred, the process repeats itself as soon as the grid of A again becomes positive. The voltage on c_1 thus rises rapidly to a maximum while c_1 is charging, and falls off slowly during its discharge. The result is a saw-tooth wave, which is the wave-form required for the oscillograph time-base.

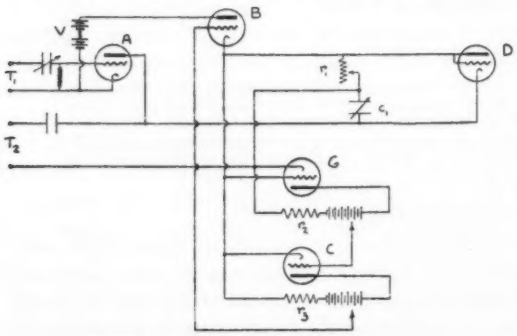


FIG. 1. *Schematic diagram of sweep circuit.*

Tube A is employed in the circuit in order that the saw-tooth wave shall be synchronized with the wave being analyzed. It is found in practice, however, that in most cases sufficient synchronization is obtained by connecting one wire from T_1 to a wire from T_2, so that tube A may be taken out and the plate and filament terminals directly connected. The circuit is then made up simply of tube B, through which c_1 charges, tube D, through which it discharges, and the amplifier Gr_2Cr_3 which controls tube B.

Operation of Circuit

As an example of the results obtained by the use of this device in connection with the Von Ardenne oscillograph tube, Plate I is shown. The first figures in each case give the frequency of the wave under examination, and the second the time of photographic exposure. The two photographs taken at 43,200 cycles are included to show that the number of waves in a single sweep may be altered at will by controlling the time of discharge of the condenser. This is accomplished by altering the value of c_1 or changing the filament current of D.

The return sweep of the cathode-ray spot becomes more noticeable at the higher frequencies, because the time required for c_1 to charge is equal to one or two periods of the wave being examined. This could be improved by a more careful design of the amplifier, as at high frequencies the one used became rather inefficient. For this reason, in order to make B sufficiently negative during the discharge of c_1, the bias on the grid of B had to be made more negative while c_1 was charging, and therefore B did not pass sufficient current to charge c_1 quickly enough.

Reference

1. BEDELL, F. and REICH, H. J. J. Am. Inst. Elec. Eng. 46: 546-549. 1927.

500 PER SEC., EXP.= 11 SEC.
43,200 PER SEC., EXP.= 10 SEC.
43,200 PER SEC., EXP.=10 SEC.
102,000 PER SEC., EXP.=16 SEC.
149,000 PER SEC., EXP.= 16 SEC.
213,000 PER SEC., EXP.=14 SEC.

Example of figures obtained at various frequencies.

A NEW METHOD OF PREPARING COLLOIDAL SILVER AND GOLD BY MEANS OF A CONTINUOUS HIGH FREQUENCY ELECTRICAL DISCHARGE[1]

By A. N. Fraser[2] and J. Gibbard[3]

Abstract

A new method of preparing metal sols, by the use of a continuous high frequency electrical discharge of sufficient intensity to produce a sinusoidal spark, is described. Stable yellow colloidal silver and purple colloidal gold have been prepared, without a protective colloid, by this method. Chemical determinations have failed to indicate any silver oxide in the yellow silver sols.

A new method of preparing colloidal silver and gold by electrical dispersion has been developed. Bredig (1898) was the first to describe a method of producing colloids by electrical dispersion. Since that time other methods have been developed with the object of producing purer sols. Svedberg (3) in his new book, "Colloid Chemistry", thoroughly discusses this field. Most of these older methods give rise to colloids containing a great variation in particle size and also produce more or less marked changes in the dispersion medium. Thus far, the most satisfactory results have been obtained by using a high frequency spark.

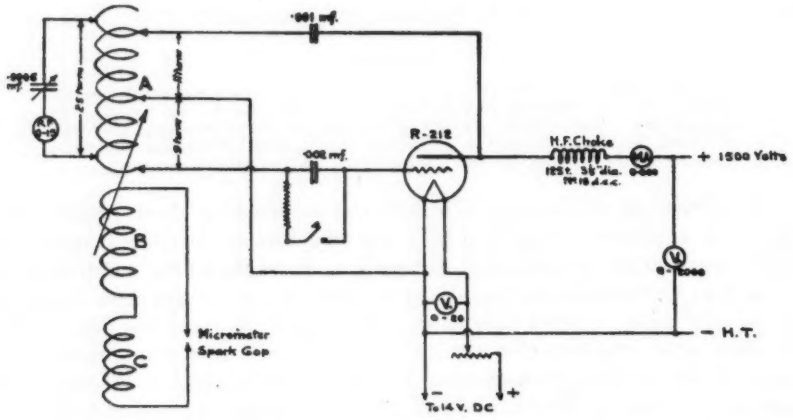
High frequency undamped oscillations have been studied extensively, with considerable success, by Kraemer and Svedberg (2) and fully described by Svedberg (3). The high frequency undamped spark was produced by using a high frequency transformer in conjunction with a low frequency alternating current transformer. Since the oscillations from this type of circuit are neither continuous nor uniform, it seemed advisable to utilize the more modern methods of producing continuous, undamped, high frequency oscillations of sufficient intensity to produce a sinusoidal spark.

The following method was found to work extremely well. The high frequency potential required to maintain a discharge between electrodes immersed in redistilled water is induced into the discharge circuit by a driving circuit, employing a thermionic tube to produce the necessary high frequency sinusoidal current. The oscillator consisted of a Northern Electric type R-212 tube connected in accordance with the well-known Hartley arrangement. The high tension direct current supplied for the anode and the low tension direct current for heating the filament were obtained from motor generators. Where suitable generators are not available, alternating current might equally well be utilized by providing suitable transformers and a rectifier employing high efficiency mercury-vapor tubes.

Fig. 1 is a schematic diagram of connections to indicate the arrangement and characteristics of the circuit.

[1] *Manuscript received July 26, 1932.*
Contribution from the Radio Branch, Marine Department, Ottawa, Canada, and the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa.
[2] *Senior Radio Electrical Engineer, Radio Branch, Marine Department, Ottawa, Canada.*
[3] *Bacteriologist in the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.*

It should be stated that the parts used in this oscillator were not specially made for the purpose, but were assembled from equipment available at the Test Room, maintained by the Radio Branch of the Marine Department, Ottawa.



A - 26 Turns, 6·75 ins. dia., spaced $\frac{5}{16}$ in.
B - 77 - , 4$\frac{1}{2}$ ins. - , No. 14 d.c.c., close wound.
C - 85 - , 3$\frac{1}{2}$ ins. - , No. 18 - , - -

FIG. 1. *Schematic diagram of high frequency continuous wave generator.*

Coil *B* was inserted wholly within coil *A* in order to obtain sufficient transfer of energy to produce a discharge across the submerged gap. Coil *C* was added to bring the natural period of the discharge or secondary circuit into the range of frequencies obtainable in the driving circuit, by means of the variable condenser connected in parallel with the primary inductance.

The oscillator is first adjusted for stable operation at maximum output by varying the position of the grid and anode taps on coil *A* while noting the reading of the thermoammeter connected in series with the variable condenser. The discharge circuit is then adjusted with the gap in air until a discharge is maintained across the electrodes while they are at least 5 mm. apart. The electrodes are then adjusted to a gap of from 1 to 2 mm., immersed in a beaker of redistilled water and the oscillator energized. A continuous non-explosive discharge now takes place between the electrodes.

Within a relatively few moments after sparking, the dispersed metal may be readily seen forming a layer in the upper part of the distilled water above the electrodes. If sparking is carried on too long the water becomes very hot and the color of the sol changes. It is, therefore, advisable to use an ice bath to maintain a low temperature.

The size of particles seems to depend upon the nature of the spark, type of discharge electrodes, temperature of the water, hydrogen ion concentration, and many other factors. So far as the electrodes are concerned, it was found

that the most satisfactory results were obtained with knife edges about 5 mm. long. By this means, the size of the spark remains more uniform, since there is merely a change of its position at the knife edges, instead of a change in length as the metal is dispersed. When flat-faced electrodes are used, a bubble of gas collects between them, and then, instead of having the spark pass through the water, it passes through the gas, with the result that the metal is dispersed in larger particles. If, on the other hand, fine wire electrodes are used, the length of the gap increases as the metal is dispersed or melted, giving unsatisfactory results.

Colloidal Silver

By this method, stable yellow colloidal silver can be readily prepared in pure redistilled water, without the addition of any protective colloid. By varying the experimental conditions, black, green, greenish-yellow, and orange-yellow silver sols have been prepared.

The quantity of silver dispersed has been measured in two instances. A black sol contained 16 mg. of silver per litre, while a bright yellow sol contained 2 mg. per litre. Undoubtedly more concentrated sols could be prepared, especially if a method could be developed to continuously remove the more concentrated upper layers.

As a test for the purity of silver sols, hydrogen sulphide was bubbled through a yellow silver sol and a solution of silver nitrate, each containing 1:100,000 silver. There was an immediate distinct darkening of the silver nitrate, but no change in the colloidal silver. However, it was noticed that within an hour or so the color of the silver sol was considerably reduced. It is considered significant, in the light of Kohlschütter's (1) statement that silver sols prepared by Bredig's method contain 19-52% of silver oxide, that there was no darkening of this silver sol. Silver oxide may be produced by this method of sparking, but it was not detectable as a sulphide. As a further test for the presence of silver oxide, the reaction described by Whitby (4) was applied. The author claims that this method will detect one part of silver in 25,000,000 parts of solution; preliminary determinations indicated that such is the case. When Whitby's method was used, silver could not be detected in these silver sols unless the sol was first treated with nitric acid. Conductivity determinations would probably settle this question, but unfortunately the necessary apparatus was not available.

Colloidal Gold

Red colloidal gold with a yellowish sheen has also been prepared, but thus far, only in the presence of a minute amount of gelatine as a protective colloid. In pure, freshly redistilled water, a purplish-red gold sol has been prepared and thus far appears to be quite stable. It would seem that it is mainly a question of solving technical details to prepare a red gold sol.

Conclusion

It has been found possible to prepare stable yellow silver and purplish-red gold sols in redistilled water without the presence of any protective colloid.

It would seem, therefore, that a continuous high frequency electrical discharge offers some advantages in the preparation of metal sols. Further work is in progress and will be reported in later publications.

Acknowledgment

The authors gratefully acknowledge the courtesy and assistance given by Commander C. P. Edwards of the Marine Department and Mr. E. D. Hayman of the Test Room, Radio Branch, Marine Department, Ottawa.

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THE DISTRIBUTION OF IODINE IN ALBERTA IN RELATION TO THE PREVALENCE OF GOITRE

I. IODINE IN THE WATER SUPPLIES[1]

By Osman James Walker[2]

Abstract

A preliminary study of the iodine content of the water supplies of the Province of Alberta has been made. The iodine content of the surface water is usually less than one part of iodine per billion (1000 million) parts of water. The iodine content of water from deep wells varies from 0 to 660 parts of iodine per billion parts of water. There is a relation between the iodine content of deep wells and the geological strata from which the water is derived. A goitre survey of the province is in progress and a map shows its distribution. A close relation between the iodine content of the water and the prevalence of goitre has not been revealed so far. A number of factors that may affect this relation have been discussed.

During the last ten years many investigations have been undertaken on the occurrence of iodine in waters, foods, soils and rocks. Attempts have been made to show that the prevalence of endemic goitre in a district (6, 8, 11, 14, 18, 23) is inversely proportional to the intake of iodine in water and foods. In the iodine surveys of the waters of Switzerland (5), New Zealand (12), and various parts of the United States (4, 10, 19, 24), it has been found that there is a reasonable correlation between the lack of iodine in the water and goitre.

Iodine in the Waters of Alberta

Since no systematic survey on the distribution of iodine has been made in Canada, it was decided to begin work in Alberta. It was considered advisable to start on water supplies with the intention of examining foods, soils and rocks later. At the same time information on goitre was assembled.

Since the iodine content of water is low, large samples for analysis are required. It was decided that 100 litres or 25 gal. would be the amount that would give most satisfactory results. Arrangements were made with doctors, teachers, agriculturalists, engineers and university students residing at various places in the province to obtain the samples, to evaporate them down to a volume of about a litre and to ship the prepared samples by express to the university. During the evaporation, the samples were kept alkaline by sodium carbonate and any crystals which formed were discarded. Data were obtained on the sources of samples and also information on the occurrence of goitre in the neighborhood. Table I shows the form of questions asked.

The method which was used for analysis of these samples is based on that proposed by McClendon (20, 22) and his coworkers, with a few minor modifications. The water was evaporated to dryness on a water bath. In some of the samples, where the dissolved solids were high, it was necessary to filter off and discard the crystals as they formed. A number of these residues were

[1] *Manuscript received June 20, 1932.*
Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta.

[2] *Associate Professor of Chemistry, University of Alberta.*

TABLE I

1. Dates of collecting sample.....
2. Total volume of water used.....
3. Source of sample (river, well, etc.).....
4. If well or spring state depth and whether water comes from sand, sandstone, shale, limestone or clay.

   .....
5. Ordinary use of water supply (household, animal, community, industrial, etc.)

   .....
6. Can you give me any opinion on the prevalence of goitre, both in humans and animals in your neighborhood?

   .....

   .....

Sec.....Tp.....Range.....

Address.....

Name.....

tested and it was found that their iodine content was negligible. The next step was to destroy all organic matter by heating in a muffle furnace at 450 to 500° C. for five hours. The calcine was then ground up and extracted with 95% alcohol. This removed the bulk of the iodine. The residue was then extracted with water and a separate determination on its iodine content was carried out. In many cases the extraction of iodine by alcohol was complete but in some aqueous extracts, especially when the iodine content of the water was high, appreciable amounts were found. The alcohol extract was evaporated to dryness, taken up with one or two cc. of water, made acid with orthophosphoric acid, and treated with 1 cc. of 0.1 *N* sodium sulphite to reduce any iodate to iodide, and transferred to a small separatory funnel. One cc. of sodium nitrite solution, 2 mg. per cc., was added and the volume made up to 10 cc. One cc. of c.p. carbon tetrachloride was then added. The contents of the funnel were shaken thoroughly and the carbon tetrachloride layer transferred to the cup of a Duboscq microcolorimeter and the color compared with a standard solution of iodine in carbon tetrachloride containing 0.1 mg. of iodine per cc. In the calculations it was assumed that the concentrations of iodine in the water layer (13) and the carbon tetrachloride were as 1:85. The water extract was evaporated to about 2 cc. and any crystals which appeared before or at this point were filtered off. The procedure from this point was the same as that used for the alcohol extract, except that it was sometimes necessary to increase the volume of water at the time of extraction with carbon tetrachloride owing to the high concentration of salts in the solution.

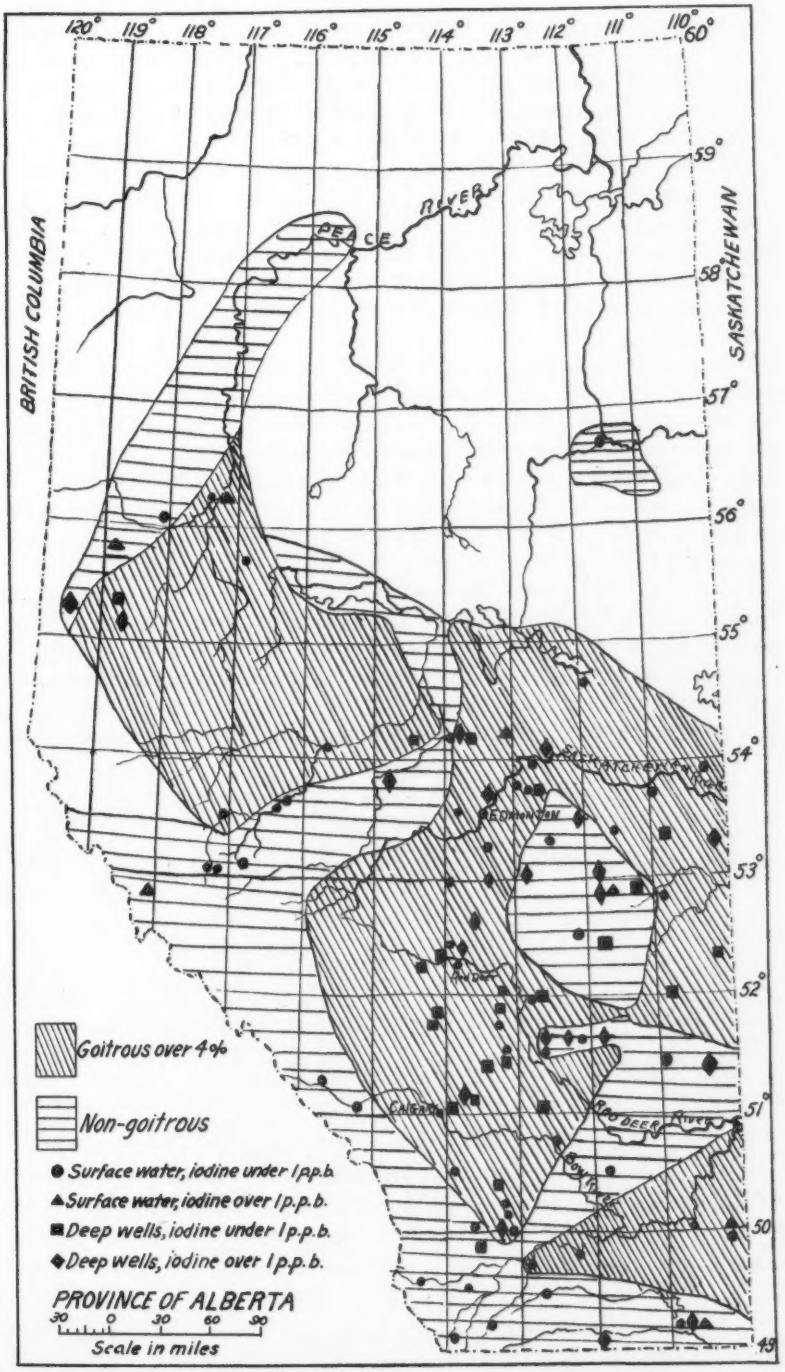

FIG. 1. *Map of Alberta showing points from which samples were obtained.*

The total iodine was equal to the sum of the values obtained for the two extracts.

One hundred and eighteen samples of water from different parts of the province were examined, the iodine content ranging from less than 0.1 parts of iodine per billion parts of water up to as high as 660 parts per billion. Table II shows the values which were obtained.

Fig. 1 is a map of Alberta showing points from which samples were obtained.

In the classification an attempt has been made to designate wells deriving water from the glacial drift as "shallow" and those deriving water from definite beds as "deep". For this reason, relatively shallow wells at Vegreville and Smoky Lake are classified as deep wells and, although fairly deep, the well at Empress is classified as a shallow well due to the thick layer of glacial drift at that point.

Referring to Table II it will be seen that surface waters, either from streams or from shallow wells, are low in iodine, containing as a rule, less than one part of iodine per billion parts of water. There are several exceptions to this rule. For example, the water from Jasper Park is high in iodine content. This is likely due to the fact that the water trickles through rocks of Precambrian age, which usually contain large amounts (2, 7) of iodine. So far it has not been possible to explain the high iodine values of surface waters from Peace River, Thorhild, Walsh and Manyberries.

The waters from deep wells are more variable in iodine content. They contain from 0 to 660 parts of iodine per billion parts of water. There is undoubtedly some connection between the iodine content of the water and that of the rock through which it passes. Fig. 2 is a geological map of the province (1), on which has been placed the location of the deep wells with their depth and the iodine content of the water. Deep wells in the area underlain

TABLE II

IODINE CONTENT OF WATER IN PARTS PER BILLION

Source	Parts per billion	Source	Parts per billion
Streams			
Banff (Bow)	0	Blairmore (Crows' Nest)	0.31
Sterco (Springs and mine water)	0	Edmonton (N. Saskatchewan)	0.33
Pigeon Lake (lake)	0	Red Deer (Red Deer)	0.35
Pincher Creek	0.1	Edson (McLeod)	0.35
Medicine Hat (S. Saskatchewan)	0.1	Gull Lake (lake)	0.38
Claresholm (Willow Creek)	0.1	Waterton (creek)	0.41
Calgary (Bow)	0.1	Evansburg (Pembina)	0.42
Cardston (creek)	0.1	Exshaw (Bow)	0.42
Fernie, B.C.	0.1	Cadomin (McLeod)	0.55
Blairmore (Crows' Nest)	0.1	Calgary (Bow)	0.64
Raymond (St. Mary)	0.12	Raymond (St. Mary)	0.96
Lethbridge (Old Man)	0.18	Taber (Old Man)	1.0
McLennan (Athabasca)	0.25	Manyberries (run-off water)	3.95
Brooks (Bow)	0.27	*Lethbridge (Old Man)	4
Bassano (Bow)	0.29	†Manyberries (reservoir)	5.2
Edmonton (N. Saskatchewan)	0.25	Jasper Park (lake)	21

TABLE II—*Continued*

Location	Depth, ft.	Parts per billion	Location	Depth, ft.	Parts per billion
Shallow wells and springs					
Champion	30	0	Craigmyle	20	0.23
Innisfree	50	0	Carbon	25	0.3
Fort Saskatchewan	22	0	Forestburg	25	0.32
High River	30	0	Manyberries	70	0.34
Three Hills	14	0	Whitecourt	25	0.46
Barons	6	0	Cadomin (spring)		0.5
Brierville	20	0	Bruderheim	20	0.6
Leduc	20	0	Fairview (shallow)		0.6
Fort McMurray	46	0	Big Valley	25	0.61
Chinook	30	0	Spruce Grove (spring)		0.66
Ryley	10	0	Empress	80	0.72
Lamont	54	0.02	Heinsville	36	0.73
Wainwright	30	0.04	Westlock	30	0.78
Carmangay (spring)		0.1	Killam (shallow)		1.77
Slawa	20	0.1	Spirit River	18	1.8
Marlboro	25	0.1	Thorhild	20	2.4
Grimshaw	60	0.1	Walsh (springs)		4.53
Delph	20	0.16	Peace River	13	32.8
Walsh	18	0.21			
Deep wells					
Elnora	150	0	Conrich	147	2.1
Olds	260	0	Lacombe	240	2.25
Standard	75	0	Ponoka	204	2.58
Vulcan	200	0	Michichi	80	2.8
Sexsmith	108	0.1	Rochfort Bridge	98	3.0
Consort	74	0.1	Conrich	127	6.5
Granum	100	0.11	Coutts	80	6.85
Ogden	176	0.12	Hanna	110	7.3
Blackfalds	75	0.32	Smoky Lake	50	7.6
Bircham	250	0.38	Westlock	125	10.37
Didsbury	160	0.41	Hythe	100	23.0
Barrhead	325	0.45	Vegreville (wells)	50–72	25.1
Big Valley	175	0.56	Lloydminster	180	25.6
Alliance	85	0.68	Camrose (wells)	130–260	26.2
Irma	84	0.74	Viking	100	29.8
Provost	170	0.76	Oliver	250	30.6
Huxley	180	0.8	Grande Prairie	400	81.0
Eckville	200	0.83	Wetaskiwin	300	102.9
Vermilion	125	1.0	Barons	232	106.6
Bruderheim	135	1.08	Wetaskiwin (wells)	272–320	164.3
Clyde	200	1.1	Manyberries	1136	222.8
Conrich	74	1.4	Killam	300	436.0
Carbon	110	1.6	Killam	243	631.6
Oyen	500	1.85	Killam	260	663

**Evaporated in a schoolroom where iodine had been prepared.*
†*A reservoir contaminated by water from a deep well rich in iodine.*

by rocks of the Paskapoo series of Tertiary age yield water which is low in iodine, usually containing less than one part per billion. An apparent exception to this rule was found in the case of the water at Wetaskiwin, but a more recent geological survey (25) definitely places Wetaskiwin, and possibly Ponoka, in the territory underlain by rocks of the Edmonton series. Deep-

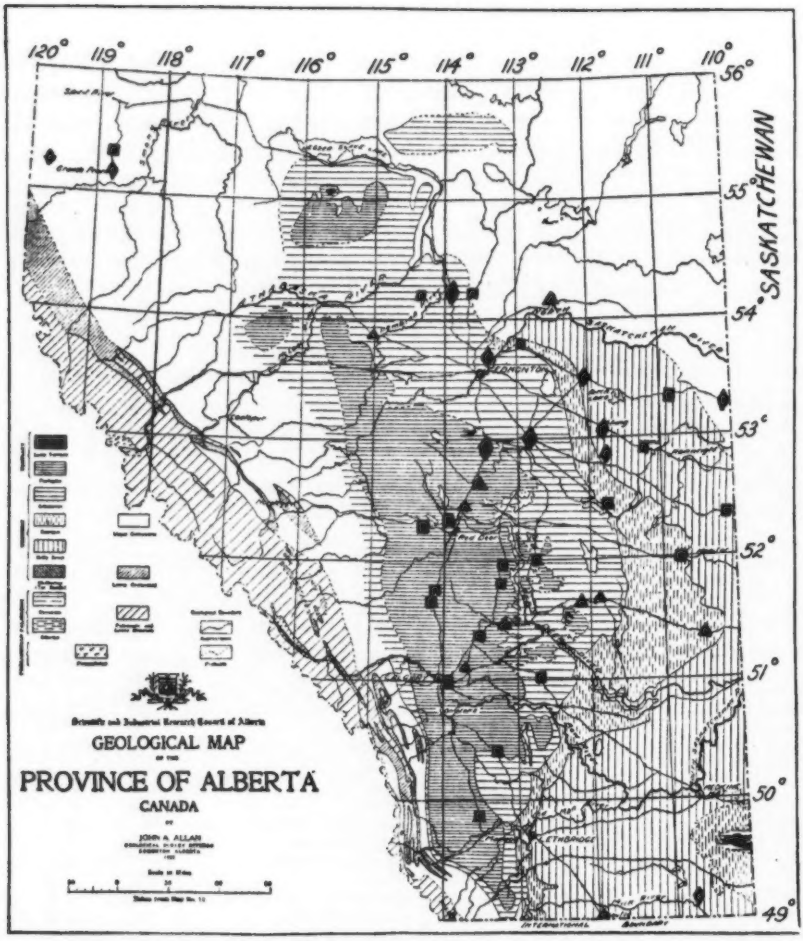

FIG. 2. *Geological map of Alberta showing the location of the deep wells and the iodine content of the water.* ■ *Deep well water; iodine, 0-1 p.p.b.* ▲ *Deep well water; iodine, 1-10 p.p.b.* ◆ *Deep well water; iodine, over 10 p.p.b.*

well waters derived from the rocks of the Upper Cretaceous, especially in the Edmonton and Belly River Series, are generally rich in iodine. Low iodine values were obtained for water from wells in the Bear Paw Series, the rocks of which are older than those of the Edmonton Series but younger than those of the Belly River Series. The highest iodine values were found in the waters of three wells north of Killam in the Belly River Series. These wells were 243, 260 and 300 feet in depth, and the iodine contents of the waters were 631.6, 663 and 436 parts of iodine per billion parts of water respectively. Other iodine values of 100 and more parts per billion were found in waters

TABLE III

PREVALENCE OF GOITRE AS REPORTED BY COLLABORATORS

Edmonton	Goitre in both humans and animals.
Barrhead	There are a considerable number of cases of goitre in the district.
Lethbridge	One hundred and one cases in Galt Hospital in five years. So goitre seems to be rather common.
Taber	More or less in the goitre belt. Goitre not uncommon.
Consort	Uncommon.
Lamont	Very little.
Sexsmith	A considerable amount among people.
Wainwright	Average in humans.
Walsh	Knows of no goitre.
Alliance	Rare.
Vermilion	In animals on school farm.
Viking	Very little goitre (well 70-100 ft.)
Cadomin	None known.
Champion	Quite prevalent in humans and animals.
Forestburg	Not many cases among humans.
Olds	"About normal." Worse in wet years than in dry years. Quite prevalent in livestock, but sporadic. Some families contain as many as five suffering from goitre.
Whitecourt	Prevalent in calves and colts. Human goitre not noticeable.
Irma	Not prevalent in either humans or animals.
Craigmyle	Six or seven cases in humans in last few years. Knows of none in animals.
Provost	Probably less than 1%.
Ogden	Some cases.
High River	Fairly prevalent in humans and animals.
Hanna	Not very prevalent.
Oliver	Not common in humans nor in animals.
Delph	Occurs occasionally in humans and animals. At least one case of goitre on farm from which the sample of water was obtained.
Pincher Creek	Some goitre in both humans and animals.
Spruce Grove	Some in calves, colts and pigs at birth. Does not believe it is prevalent in humans.
Killam	No goitre in animals in district. Some in people.
Three Hills	About 1%.
Calgary	Prevalent.
Wetaskiwin	Six cases in last six months in about half the population of the town.
Clyde	None in humans and animals.
Carmangay	Very little in animals and humans.
Ponoka	Some in humans and animals. Not a great deal.
Medicine Hat	Not very prevalent.
Barons	Five or six cases in women in radius of 10-15 miles. Some in livestock.
Barons	Some in humans.
Manyberries	No known cases.
Slawa	Apparently rare.
Smoky Lake	Not prevalent in animals. Considerable adolescent goitre in humans.
Brierville	Rare.
Westlock	Moderate—about 10% in humans.
Vegreville	Very little in humans and animals.
Claresholm	Rare.
Blairmore	Not prevalent in humans. Some in cattle.
Rochfort Bridge	Occasionally appearing in calves and lambs.
Bruderheim	Knows of five cases in humans.
Westlock	Common among sheep.
Gull Lake	Quite prevalent.
Lacombe	Quite prevalent.
Sterco	None.
Marlboro	50%—high.
Wetaskiwin	Quite prevalent.
Calgary	Fairly common among young people.
Exshaw	No cases.
Edson	Knows of no cases.
Pigeon Lake	No goitre among humans.
Brooks	Not unduly prevalent.

TABLE III—*Continued*

Waterton Lakes	A few cases in humans. No cases in animals noticeable.
Leduc	Fairly prevalent in humans.
Thorhild	None in this district. Some in humans in neighboring school district.
Grimshaw	Ten per cent or higher in humans. Quite prevalent in animals.
Coutts	No cases in children and animals known. Some in adults of middle age.
Peace River	Fairly prevalent, especially along inland waterways.
Eckville	Prevalent among humans, rare among animals.
Grande Prairie	Not common in the town.
Fairview	Very little among humans. Prevalent among animals.
Cardston	Quite prevalent among humans. Unable to state about animals.
Michichi	Not prevalent. Only a few cases in the district.
Heinsville	Knows of six cases in humans—probably more.
Jasper	Not prevalent.
Big Valley	Prevalent. Several cases in town.
Oyen	Rather rare in lambs and calves. Fifteen cases in Cereal in eight years.
Didsbury	Very prevalent among children.
Fernie	Fairly common, especially among older people.
Standard	Two cases known within eight miles.
Raymond	Rare (previously, water taken from deep well).
Huxley	Several cases in the neighborhood (two on this farm).
Spirit River	Very rare. Eleven out of forty children in a school nearby have the disease.
Fort McMurray	None.
Conrich	None within four miles.
Granum	Some in the district.
Evansburg	Ten cases in four years.
Blairmore	None in humans and very little in animals.
Chinook	Not prevalent.
Hythe	Not common.
Killam	None on farms with deep wells.
Red Deer	Thirty-two cases in the town schools (549 students, 5.8%). Quite prevalent in animals.
Bentley	Seven cases in 98 school children = 7%.
Bowden	Sixteen cases in 109 school children (14 being girls) = 15%.
McLennan	Some.

from wells at Wetaskiwin and Barons in the Edmonton Series and also at Manyberries in the Belly River Series. Values between 25 and 100 parts per billion were found in water from wells at Viking and Vegreville in the Belly River Series, Oliver and Camrose in the Edmonton Series, and Grande Prairie and Lloydminster in the undivided series in the Upper Cretaceous. The deepest well from which a sample was tested has a depth of 1136 ft. and is located near Manyberries. This is an artesian well owned by the Dominion Range Experimental Station in the southeastern corner of the province.

As previously mentioned, three deep wells were sampled near Killam. Samples of water were also obtained from three deep wells in the Conrich district northeast of Calgary. The iodine contents of the three samples were similar to one another although the values were all higher than expected from their geological position.

It will be noted in Table III and Fig. 1 that a number of samples were obtained from the same locality but were derived from dissimilar sources. The water from a river and a spring at Cadomin contained almost identical amounts of iodine. Surface and near-surface waters from the vicinity of Manyberries, Bruderheim, Barons, Westlock and Killam were much lower in iodine content than the water from the deep wells in the same district. In

many other districts there are also varied sources of water supply but these have not been investigated as yet.

By referring to the maps in Figs. 1 and 2 it will be seen that the waters of some districts have not been tested and some need a closer examination. It has been difficult to find collaborators for collecting and preparing the samples in districts which are sparsely settled and isolated from the centres of population. It is hoped that the survey may be further advanced in the near future.

Goitre Survey

Nearly every collector of the water samples for the iodine test furnished information on the prevalence of goitre in their districts. In many cases they consulted with the local physicians and veterinarians so that the data are fairly reliable. The information so obtained is presented in Table III.

Valuable information was also obtained from the Travelling Health Clinics which were sent to various rural centres in the province by the Department of Health during the summers of 1929, 1930 and 1931. Data for 1931 are not yet available. Table IV shows the information on goitre collected by the clinics during 1929 and 1930. The health clinics operated as follows. A centre was chosen and the nurses examined the children in the schools in the neighborhood. Those who required any medical or dental attention were asked to report at the clinic. Each clinical centre represented about 12 school districts. Column 2 shows the number of children examined by the physicians at the centre; column 3 shows the number examined by the nurses in the neighboring schools; column 4 contains the number of children having thyroid enlargements; and column 5 is the percentage of goitre in comparison to the total number examined by the nurses. It will be noted that for some of the centres no statistics are given regarding the number of children examined by the nurses. These statistics are lacking because of either no examination in the schools, or because of independent examinations by district nurses or district physicians.

The percentage of goitre in children as shown in this table varies from zero to 22.5%.

Additional information has been obtained from doctors and veterinarians in various parts of the province.

By using the information from these sources, a tentative goitre map for Alberta has been compiled and appears along with the source of water samples on Fig. 1. On this map, goitrous districts represent an incidence of over 4% as indicated by the data available. It is difficult to draw definite boundaries between goitrous and non goitrous areas because of the fact that often a small district where goitre is prevalent may be surrounded by districts where thyroid enlargement is rare and *vice versa*. There is little evidence of goitre in the mountain region among humans although there is some among animals. Goitre is not prevalent near the southern boundary of the province. There is a great deal of goitre in a strip of territory between Edmonton and Calgary. Goitre is prevalent in the irrigated districts of the southern part of the province and also in the north in part of the Peace River territory where drinking

water comes chiefly from rain and snow. Some of the newly settled districts not yet reached or just recently served by railways are more goitrous than those in the same vicinity but settled for a longer time.

TABLE IV

NUMBER OF THYROID CASES AMONG CHILDREN PRESCRIBED FOR BY THE ALBERTA TRAVELLING CLINIC

Clinic centre	No. of children examined by physicians	No. of children examined by nurses	No. with goitre	% with goitre	Clinic centre	No. of children examined by physicians	No. of children examined by nurses	No. with goitre	% with goitre
1929									
Pincher Creek	97	223	5	2.2	Kinuso	57	69	3	4.3
Dunstable	43		3	7	Strathmore	154	202	24	11.9
Coutts	99	218	4	1.8	Bulwark	75	307	4	1.3
Markerville	104	194	13	6.7	Hillspring	257	307	5	1.6
Cessford	158	164	2	1.2	Tawatinaw	86	204	11	5.4
Jarvie	80	89	2	2.2	Plamondon	164	346	11	6.1
Grouard	91	196	4	2	Lac la Biche	127		10	
Millet	152	188	14	7.4	Breton	52	137	8	5.8
Sundre	79	205	13	6.3	Oyen	80	180	4	2.2
Diamond City	197	390	11	2.8	Pendryl	68	78	8	10.3
Naco	101	98	3	3	Slave Lake	38	69	0	0
Youngstown	49	189	2	1.1	Wanham	72		5	7.0
Collholme	79	221	4	1.8	Clyde	100	211	6	2.8
New Brigden	149	218	7	3.2	Sangudo	100	298	2	.6
Didsbury	112	188	26	13.8	Mountain View	101	240	1	.4
Mosside	83	130	5	3.8	Onoway	154	270	6	2.2
Wabamun	99		2	2	Mirror	200	386	8	2.1
Darwell	78	123	4	3.3	Clive	213	359	15	4.2
Mayerthorpe	154	137	8	5.8	Eastburg	44		1	2.3
Cherhill	89	149	3	2					
1930									
Mannville	226	357	39	10.9	Rumsey	144	130	11	8.5
Paradise Valley	126	306	19	6.2	Irvine	80	154	11	7.1
Metiskow	175	191	20	9.5	Whitla	64	80	6	7.5
Lakesend	120	120	27	22.5	Orion	119	135	10	7.7
Henaruka	155	150	10	6.7	Wrentham	60	249	3	1.2
Altario	140	268	10	3.7	Del Bonita	64	89	2	2.2
Sibbald	159	375	7	1.9	Travers	147	266	5	1.9
Sunnydale	124	195	5	2.6	Fort Vermilion	155		4	2.6
Sunnynook	115	79	9	11.4	Notikewin	127		4	3.3
Peers	65	100	2	2	Grimshaw	82		8	9.8
Strome	107	610	11	3.6	Whitelaw	138		5	3.7
Killam	97		11		Spirit River	93		2	2.1
Halkirk	110	248	2	0.8	Valhalla	150		22	14.7
Byemoor	126	196	21	10.7	Beaver Lodge	153	154	12	7.8

Relation Between Iodine in the Water and Prevalence of Goitre

In correlating the prevalence of goitre in Alberta with the lack of iodine in the water supply, the relation does not hold as closely as has been found in other countries. For example, in the mountain region where the iodine content of the water is low, goitre is rare, but in the foothills and neighboring districts

to the east where the iodine content of the water is also low, goitre is endemic in both humans and animals.

Iodine as a prophylactic for goitre has been known since the early work of Chatin (3) and is now used to a very large extent in goitrous countries in the form of iodized salt. This is not only true in districts where iodine surveys have been made but also in other districts. Table V, for example, shows the amount of the different varieties of salt sold by a wholesaler in the Edmonton district.

TABLE V

AMOUNTS OF SALT SOLD BY A WHOLESALER IN THE EDMONTON DISTRICT

	Non-iodized, lb.	Iodized, lb.
Salt in 50-lb. bricks	800,000	250,000
Salt in bags	1,600,000	
Salt in cartons	14,400	72,000

These amounts represent approximately one-tenth of the total consumption of salt for the province. Salt in bricks is used for animals while other forms of salt are for household and industrial uses. In some places on this continent (16, 26) the drinking water is periodically treated with sodium or potassium iodide and the amount of goitre has been thereby materially reduced.

The water supply of a region is obtained from many diverse sources. Even in a small area the water may be obtained from streams, or shallow wells, or from deep wells that tap strata at different depths below the surface. Water dissolves soluble salts from the soil or rock with which it comes in contact as it moves on the surface or underground. It has been shown (9, 15, 17) that soils and rocks contain variable amounts of iodine in combination. Part of this is leached out and enters the water. The amount of iodine entering the water will depend on the time the water has been in contact with the soil or rock, also on the richness of the soil or rock in iodine, and on its solubility under the conditions of leaching. For these reasons the iodine content of the water supply may be variable in one district. Plants take up varying amounts of iodine depending on the quantity of this element in the soil and the ground water. A district may obtain all of its water supply from deep wells containing a high iodine content (21) but the plants will be low in this element due to the fact that the surface water with which they come in contact is low in iodine. In some districts practically all of the food is brought in from outside sources. All of these factors must be kept in mind if one wishes to draw any relation between the amount of iodine and the prevalence of goitre.

The Province of Alberta has an area of about 260,000 square miles and contains a great variety of topographic features. The soil on the surface is largely composed of glacial drift. It is underlain by rocks of many ages, ranging from Precambrian to the later Tertiary. In the mountain districts most of the food is imported but on the plains a great deal is locally grown. Iodized salt, as shown, is used more or less extensively. Drinking water supplies are obtained from streams, shallow wells, deep wells and even from rain and snow. The population of Alberta consists of people of many nationalities who practise different methods of cooking. There will undoubt-

edly be varying amounts of iodine lost during the cooking processes. This factor has been discussed by Stiner (27) in attempting to correlate goitre with iodine in parts of Switzerland. With the variety of conditions prevailing in Alberta the difficulty which is met with in correlating iodine with goitre will be understood.

Further studies on the distribution of iodine in Alberta are being undertaken and it is hoped that more light will be shed on the relation of this element to the occurrence of goitre.

Acknowledgment

The author wishes to express his thanks to Dr. M. R. Bow, Deputy Minister of Health, who furnished the statistics gathered by the Travelling Clinics; to the members of the Geology Department of the University of Alberta who gave special details on geology; to the officials of the Provincial Laboratory who assisted in the transportation of the water samples; and to all of those persons who collected and prepared the water samples throughout the province.

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THE KINETICS OF THE OXIDATION OF GASEOUS ACETALDEHYDE[1]

By W. H. Hatcher[2], E. W. R. Steacie[3] and Frances Howland[4]

Abstract

The kinetics of the oxidation of gaseous acetaldehyde have been investigated from 60° to 120° C. by observing the rate of pressure decrease in a system at constant volume. A considerable induction period exists, during which the main products of the reaction are carbon dioxide, water, and formic acid. The main reaction in the subsequent stages involves the formation of peroxides and their oxidation products. The heat of activation of the reaction is 8700 calories per gram molecule. The indications are that the reactions occurring during the induction period are heterogeneous. The subsequent reaction occurs by a chain mechanism. The chains are initiated at the walls of the reaction vessel, and are also largely broken at the walls.

Introduction

A kinetic study of the oxidation of many organic compounds in the gaseous state has led to the conclusion that these reactions occur by a chain mechanism. Under such conditions a large number of molecules react for each primary step which occurs, and hence the order of the reaction is abnormal and not necessarily integral, and the rate of reaction differs from that which might be expected from the heat of activation. Any factor which influences the propagation of the chains will have a pronounced effect on the rate of reaction, and hence such reactions are often influenced by foreign gases, inhibitors, and the walls of the container. In general, the characteristics of such a reaction will depend on the length of the chains, and on the manner in which they are initiated, continued, and broken.

Hinshelwood and his coworkers have investigated the oxidation of ethylene (15), benzene (9), methane (10), methyl alcohol (10), and formaldehyde (10). All these reactions proceed by a chain mechanism, and in each case an induction period occurs, which is explained as due to the formation of unstable peroxides which serve as centres from which chains are propagated.

It seems advisable to extend such investigations to the study of the oxidation of other organic compounds, and the present paper deals with the gas-phase oxidation of acetaldehyde. It is unnecessary at this point to refer to the extensive literature on the oxidation of acetaldehyde, but such details as have a direct bearing on this investigation will be mentioned in the appropriate place.

Apparatus

The reaction was investigated by introducing acetaldehyde-oxygen mixtures into a heated vessel and observing the rate of change of pressure as the reaction proceeded.

The apparatus employed was similar to that used by one of the authors for

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the oxidation of acetone (14), and is illustrated in Fig. 1. The reaction bulb, *A*, was contained in an electric furnace, *B*. The temperature was measured by a standard thermometer, *D*, and the furnace was hand-regulated by rheostats to within 1°C. The capillary tubing between the reaction bulb and taps 1, 4, and 5 was wound with nichrome wire and maintained at the temperature of the furnace.

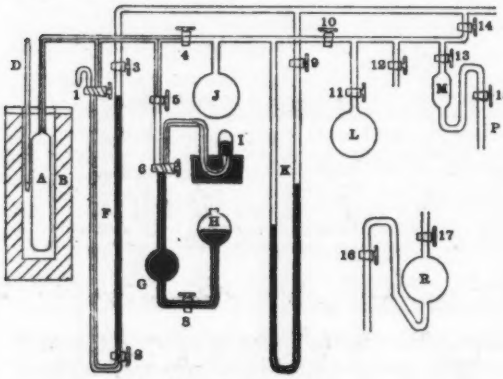

FIG. 1. *Apparatus.*

The capillary manometer, *F*, was not heated. A small amount of air could be admitted to the manometer through tap 1. This served as a buffer to keep the mixture out of contact with the mercury surface. The necessity of this precaution has been shown in a previous publication (11).

Oxygen and acetaldehyde were stored in the containers *L* and *M*, and mixtures of the desired proportions were made by admitting these as gases to the mixing bulb, *J*, and observing their partial pressures by means of the manometer, *K*.

The oxygen employed was obtained from cylinders and was dried over phosphorus pentoxide. Acetaldehyde was prepared from a high grade of paraldehyde by distillation with a few drops of concentrated sulphuric acid. It was redistilled in the presence of carbon dioxide immediately before each experiment, and was drawn over directly from an ice-cooled receiver to the acetaldehyde reservoir through tube *P*. Thus the acetaldehyde used was in contact with oxygen only for a very short time before reaching the reaction bulb. The importance of this has also been pointed out in a previous communication.

The second reaction bulb, *R*, was used instead of the simple bulb for those experiments in which the condensable products of the reaction were investigated.

Experimental Procedure

Prior to each experiment the reaction bulb was evacuated for a few hours while kept at about 350°C. This was found to be necessary in order to remove material from the walls which affected the rate. The furnace was then brought to the desired temperature, and some of the gas mixture in *J* was admitted to the reaction bulb through tap 4, tap 1 having been previously closed. Tap 4 was then closed and tap 1 opened, and the initial pressure of the mixture was read on the capillary manometer. A small quantity of air at a pressure slightly greater than that of the mixture had been previously admitted to the manometer to serve as a buffer. Tap 1 was opened only momentarily for the pressure readings, which were made at regular intervals during the course

of the reaction. The correction for capillary depression was made automatically by taking the zero reading of the manometer as the position of the mercury when the system was evacuated.

By means of the bulbs *G* and *H* samples of gas for analysis could be pumped out of the reaction vessel and collected over mercury in the tube *I*. For those experiments in which it was desired to obtain an aqueous solution of the condensable products for analysis, the reaction bulb *R* was substituted for *A*. When the reaction had reached the desired stage, the furnace was removed, tap 17 was closed, the exit tube was immersed in distilled water, and on opening tap 16 water was sucked into the reaction bulb. The solution thus obtained, together with four washings, was collected and made up to 500 cc.

The Products of the Reaction

The quantity of condensable products, including unchanged acetaldehyde, was estimated from the pressure change obtained on cooling the products in the reaction bulb to −78°C. While the reaction bulb was thus cooled, samples of gas were removed for analysis to a modified type of Bone-Newitt gas analysis apparatus.

The aqueous solution of the reaction products was tested quantitatively for the following: (a) total acidity with 0.02 *N* sodium hydroxide; (b) peracids and peroxides with neutral potassium iodide and acidified potassium iodide respectively, and subsequently with 0.02 *N* sodium thiosulphate by the method of Clover and Houghton (4); (c) total reducing agents with alkaline permanganate according to the method of Cameron and McEwan (3); (d) a portion of the solution was heated for from four to five hours at 60°C. to remove unchanged acetaldehyde, and the above tests were repeated. The reducing agent left was assumed to be formic acid.

Previous work on the oxidation of acetaldehyde has shown that under various conditions many different products can be obtained. Thus an aqueous solution of acetaldehyde was oxidized by means of cerium sulphate in the presence of dilute sulphuric acid to glycollic aldehyde and glyoxal, together with large quantities of formic acid and carbon dioxide (5). Oxidation with aqueous hydrogen peroxide gave a complex peroxide, which reacted further to give acetic acid and water (13). An acid solution of potassium permanganate yielded acetic acid alone, whereas an alkaline solution produced acetic acid, oxalic acid, and carbon dioxide, the relative proportions of the products depending on the alkalinity of the solution (8). Fewer products have been found as a result of the oxidation of gaseous acetaldehyde by means of air or oxygen, except in the presence of catalysts (2, 6, 12, 16, 17).

All investigators who have studied the oxidation of acetaldehyde by oxygen agree that the primary product is peracetic acid. They do not agree, however, on the mechanism by which it is formed, or the manner in which it subsequently reacts. Complete analyses of the products of the oxidation have never been reported.

The products found in the present investigation are as follows:—peroxygen compounds, formic and acetic acids, and carbon dioxide in small quantities

in the earlier stages of the reaction and in larger quantities together with carbon monoxide in the later stages. It is thus apparent that peracetic acid is not the sole product of the oxidation even in the earliest stages of the reaction. It is of interest to compare the quantities of the products formed at various stages of the reaction, in order to discover the significance of the pressure change. In Table I the products and pressure decreases are tabulated in terms of the percentage of the initial amount of acetaldehyde present.

TABLE I

THE PRODUCTS OF THE REACTION*

1 $CH_3CHO+1.5\ O_2$

Pressure decrease	Oxygen used	CO_2 formed	Condensable, including acetaldehyde	Total acids	Formic acid	Peroxygen compounds
1.6	20.2	5.9	113	—	—	—
10.0	29.8	6.6	113.4	20	15	6
22.1	40	7.1	111	—	—	—
30	—	—	—	50	17	12
48	62.3	6.4	108	—	—	—
50	—	—	—	60	13	8
70	—	—	—	80	0	11
90	—	—	—	90	0	11

Temperature, 60°C.

It is apparent that up to a 50% pressure decrease constant amounts of carbon dioxide, formic acid, and peroxygen compounds are formed at all stages. The amount of oxygen used is remarkably large at the beginning of the reaction and finally increases in a manner corresponding to the increase in total acidity.

The products are not appreciably affected by a variation in temperature. Analytical results for 78°C. are given in Table II.

TABLE II

THE PRODUCTS OF THE REACTION AT 78° C.

1 $CH_3CHO+1.5\ O_2$

Pressure decrease	Oxygen used	CO_2 formed	Condensable, including unchanged acetaldehyde
3.2	25.0	5.5	116.0
19.7	38.0	8.8	109.3
44.4	68.5	6.7	117.5
46.4	70.0	3.9	119.9

Increasing the surface of the reaction vessel has a marked effect on the amount of carbon dioxide formed, and of oxygen used. As shown by Table III, a constant quantity of carbon dioxide is again formed at every stage of the reaction, and a constantly increasing quantity of oxygen is used as the reaction progresses.

TABLE III

ANALYSIS OF PRODUCTS FORMED IN PACKED BULB

Temperature, °C.	Pressure decrease	Oxygen used	CO_2 formed	Condensable, including unchanged acetaldehyde
60	0.20	26.0	9.0	117.5
60	0.75	42.0	18.0	123.0
60	23.2	55.5	14.0	118.0
60	46.5	67.5	15.6	105.7
60	76.0	84.5	11.7	97.5
78	0.56	34.0	19.0	115.0
78	50.0	84.2	24.2	110.0

It may therefore be concluded that at the beginning of the reaction, before the pressure has decreased to an appreciable extent, some carbon dioxide and formic acid are formed, the amount depending mainly on the surface of the reaction bulb, and to some extent on the temperature. These substances apparently do not continue to form during the later stages of the reaction, or at all events if they do form they break up again at an equal rate. The subsequent reaction, *i.e.*, the one which is accompanied by a pressure decrease, is an oxidation reaction since a constantly increasing amount of oxygen is used up during the pressure change.

The Pressure Change Accompanying the Reaction

As indicated before, the reaction is accompanied by a pressure decrease, the rate of which reaches a maximum only after a considerable time, especially at low temperatures. The maximum pressure decrease reached varies from 50 to 100% of the initial partial pressure of acetaldehyde, the percentage depending on the amount of oxygen present. At 60°C., however, the rate of pressure decrease does not reach zero but attains a constant low value after 100% decrease is reached, which is undoubtedly due to partial condensation of the products of the reaction. At higher temperatures after passing through a minimum the pressure begins to increase again. The present investigation, however, is concerned only with the first phase of the reaction, *i.e.*, the part which is accompanied by a decrease in pressure.

Oxidation of acetaldehyde according to the equations given below would result in the following pressure changes, expressed as percentages of the initial partial pressure of the acetaldehyde.

	Pressure change
$CH_3CHO+0.5\ O_2=CH_3COOH$	50% decrease
$CH_3CHO+1.5\ O_2=2\ HCOOH$	50% decrease
$CH_3CHO+1.0\ O_2=CH_3CHO.O_2$	100% decrease
$CH_3CHO+2.5\ O_2=2\ CO_2+2\ H_2O$	50% increase
$x CH_3CHO+\ y\ O_2=$Other peroxides	100% decrease, or more.

Since the pressure decrease always tends to a value of 100%, a relatively stable peroxygen compound must be the major product in the early stages of the oxidation. To decide what other reactions occur simultaneously other considerations must be taken into account.

Table IV shows the maximum pressure decreases reached under various conditions.

TABLE IV

MAXIMUM PRESSURE DECREASES

Temperature, °C.	$\frac{O_2}{CH_3CHO}$	Pressure decrease as per cent of partial acetaldehyde pressure	$\frac{O_2/CH_3CHO}{\%\text{ Pressure decrease}}$
80	1.20	82	0.0147
100	1.17–1.25	78	0.0155
100	1.34	88	0.0153
100	1.40–1.50	95	0.0153
100	1.50	100	0.0150
120	1.18–1.26	77	0.0158
120	1.32–1.36	81	0.0165
120	1.40–1.48	88	0.0164
120	1.53–1.57	98	0.0158
120	1.60–3.00	100	0.0160

It is thus apparent that if 1.6 times as much oxygen as acetaldehyde is used, a pressure decrease of 100% will always be obtained at 120°C., and this is also approximately true for 100° and 80°C. If a smaller amount of oxygen is used, the ratio $\frac{\text{oxygen/acetaldehyde}}{\%\text{ pressure decrease}}$ is constant for any given temperature. The maximum pressure decrease therefore depends almost entirely on the relative quantities of the reactants. The overall equation for the oxidation may therefore be written

$$CH_3CHO + 1.6\ O_2 = \text{Products.}$$

The Mechanism of the Reaction

It has already been suggested by the authors and others that the major part of the reaction involves the combination of one molecule of acetaldehyde and one of oxygen to form a peroxygen compound. This would involve a pressure decrease of 100% as required, but it obviously expresses only one of several reactions which occur, since, as already shown, other products besides peroxygen compounds are formed, and more than one molecule of oxygen is used per molecule of acetaldehyde.

It is possible at this point to suggest what reactions occur (a) during the induction period, and (b) during the subsequent stages, on the basis of products found, oxygen used, and pressure change.

The following data were obtained in a typical experiment carried out in a reaction vessel packed with broken glass.

Temp., °C.	Initial acetaldehyde pressure, cm.	O_2/CH_3CHO	Pressure decrease	O_2 used	CO_2 formed	Condensable, including acetaldehyde
			Percentage of acetaldehyde partial pressure			
60	8.7	1.96	0.65	42	18.2	123.5

Assume that the following reactions occur simultaneously:

(1) $CH_3CHO+2.5\ O_2=2\ CO_2+2\ H_2O$,
(2) $CH_3CHO+1.5\ O_2=2$ HCOOH.

To explain the products formed, these two reactions must occur to the following relative extents:

(1) $9\ CH_3CHO+22.5\ O_2=18\ CO_2+18\ H_2O$,
(2) $13\ CH_3CHO+20\ O_2=26$ HCOOH.

The pressure change accompanying this is 2.5%, and the condensable products are 26 HCOOH, 78 CH_3CHO, and 18 H_2O, a total of 122%. No products other than formic acid and water could account for an amount of condensable products larger than 100% of the acetaldehyde used, and at the same time explain the very slight pressure change.

The above mechanism is confirmed by a large number of analytical results. The data for a typical experiment in an unpacked bulb at 60°C. are given below.

	Observed, %	Calculated, %		Observed, %	Calculated, %
Formic acid	15.0	(15.0)	Pressure decrease	1.6	2.2
Carbon dioxide	6.0	(6.0)	Condensable		
Oxygen used	20.2	18.7	products	112.7	110.5

We may therefore consider the mechanism of the processes occurring during the induction period to be established.

For the later stages of the reaction good agreement with experimental results is obtained if we assume that a mixture of the two peroxides, $CH_3CHO.O_2$ and $(CH_3CHO)_2O_3$, is formed. Table V gives a comparison of observed analyses with those calculated in this way. In every case the experimental values lie between those calculated for the two peroxides.

TABLE V

PRODUCTS OF THE LATER STAGES OF THE REACTION

Pressure decrease, %			Condensable products, %		
Observed	Calc. for AcO_2	Calc. for Ac_2O_3	Observed	Calc. for AcO_2	Calc. for Ac_2O_3
Temperature, 60° C.					
23.2	21	33	118	120	108
46.5	34	53	106	120	100
76.0	50	81	97	120	90
Temperature, 78° C.					
50.0	42	72	110	119	88

NOTE:—*Packed reaction vessel. Partial acetaldehyde pressure, 13.5 cm. Oxygen/acetaldehyde ratio, 1.90.*

Equally good agreement is obtained in other cases. It should, however, be pointed out that the suggested mechanism for this part of the reaction is by no means the only possible one.

The Velocity of the Reaction

The use of the pressure change as a measure of the rate of oxidation of acetaldehyde is justified by two facts. In the first place the amount of oxygen used increases regularly as the pressure decreases, as shown by Table II. In addition, the form of the pressure-time curves remains unaltered when the conditions under which the reaction occurs are altered. Fig. 2 shows that the curves obtained with different partial pressures of acetaldehyde and with different proportions of acetaldehyde and oxygen are affine. In a similar way Fig. 3 shows that the form of the curves is unaltered when the temperature is varied.

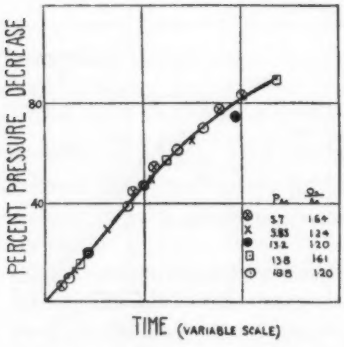

FIG. 2. *Affine curves for 120°C., showing the effect of acetaldehyde pressure and of oxygen-acetaldehyde ratio.*

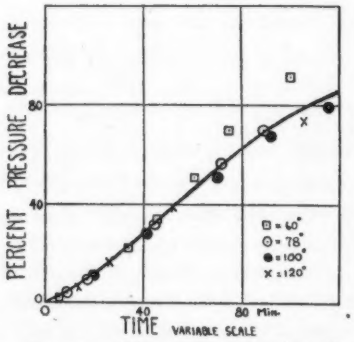

FIG. 3. *Affine curves showing the effect of temperature. Partial acetaldehyde pressure = 9.0 cm. Oxygen—acetaldehyde ratio = 1.30.*

Data for a typical run are given in Table VI.

TABLE VI

TYPICAL REACTION VELOCITY DATA

1 CH_3CHO+1.62 O_2

Time, min.	Pressure decrease, cm.	Pressure decrease as per cent of initial acetaldehyde press.	Time, min.	Pressure decrease, cm.	Pressure decrease as per cent of initial acetaldehyde press.
0	0	0	23	11.27	77.1
3	1.45	9.9	28	12.47	85.4
6	3.25	22.3	32	13.15	90.1
12.5	7.10	48.6	44	14.45	98.8
17	9.23	63.2	51	14.65	100.2

NOTE:—*Temperature, 120° C. Initial acetaldehyde pressure, 14.6 cm.*

The effect of the total pressure on the rate of reaction is illustrated by

Fig. 4, in which pressure-time curves are given for the same mixture and temperature, but for different total pressures.

As stated before, there is an induction period which is particularly noticeable at low temperatures. In order to determine the effect of various factors on the rate of reaction it is customary to compare the time for some arbitrarily defined fractional change. In the present case the times for the reaction to proceed from 20 to 40% pressure decrease have been chosen in order to eliminate the effect of the induction period.

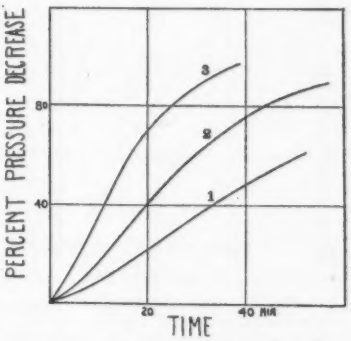

FIG. 4. *Effect of total pressure on the rate of reaction. 120°C. $O_2/Ac = 1.60$*

Curve No.	*1*	*2*	*3*
Acetaldehyde press., cm.	*5.8*	*9.6*	*14.6*

Table VII shows the effect of temperature, pressure, and the relative proportions of the reactants on the value of $T_{40} - T_{20}$. It will be seen from the table that the rate of reaction increases rapidly as the partial pressure of the acetaldehyde increases, but is independent of the partial pressure of oxygen. The order of the reaction with respect to acetaldehyde can be found in the usual way by plotting $\log P_{Ac}$ against $\log(T_{40} - T_{20})$ and measuring the slope of the line. The values thus obtained are given in Table VIII.

TABLE VII

THE EFFECT OF VARIOUS FACTORS ON THE RATE OF REACTION

Temp., °C.	P_{ac}, cm.	O_2/Ac ratio	$T_{40} - T_{20}$, min.	Temp., °C.	P_{ac}, cm.	O_2/Ac ratio	$T_{40} - T_{20}$, min.
120	18.3	1.21	6.0	120	9.6	1.95	8.0
120	14.7	1.18	6.5	120	5.8	1.64	14.0
120	12.8	1.20	7.5	100	14.8	1.17	18
120	5.5	1.26	11.0	100	11.5	1.25	19
120	15.1	1.34	7.0	100	8.4	1.18	27
120	10.1	1.32	7.0	78	18.8	1.18	24
120	15.2	1.43	6.5	78	13.3	1.20	30
120	9.3	1.48	7.0	78	8.2	1.23	47
120	5.4	1.42	14.0	60	18.9	1.25	29
120	15.2	1.57	7.0	60	10.9	1.28	76
120	9.2	1.53	9.0	60	5.75	1.30	110
120	14.6	1.62	5.0				

TABLE VIII

THE ORDER OF THE REACTION

Temperature, °C.	120	100	78	60
Order	1.47	1.7	1.7	ca. 1.7

The Temperature Coefficient

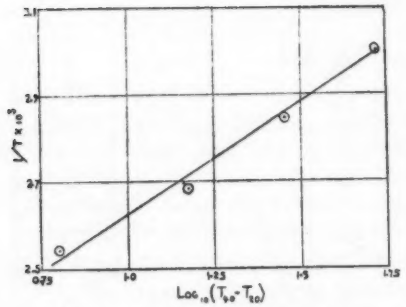

FIG. 5. *The temperature coefficient of the reaction. $O_2/Ac = 120$. Partial acetaldehyde pressure = 15.0 cm.*

In Fig. 5 the reciprocal of the absolute temperature is plotted against log $(T_{40} - T_{20})$. From the slope of the line the heat of activation is found to be approximately 8700 ± 700 calories. The data from which Fig. 5 was constructed are given in Table IX.

The Effect of the Surface of the Reaction Vessel

The effect of the surface was investigated by adding glass to the reaction vessel. In one series of experiments Pyrex tubing having an inner diameter of about 3 mm. was broken into lengths of 1-2 cm. and used. The total surface in this case was about five times the original surface. Two samples of crushed glass were also used, both of which increased the surface to about ten times its original value. The effect of the increased surface on the reaction velocity is shown in Table X.

TABLE IX

THE TEMPERATURE COEFFICIENT OF THE REACTION

Temperature, °K.	393	373	351	333
$T_{40} - T_{20}$, min.	6.5	15.1	27.9	51.9

Initial partial pressure of acetaldehyde, 15.0 cm.

TABLE X

THE EFFECT OF SURFACE ON THE RATE

Temperature, °C.	Total surface / Original surface	Sample of glass, no.	Rate / Original rate
120	5	1	3.36 3.93 4.09
100	5	1	3.83 4.00
120	10	2	4.60 4.42
120	10	3	4.49 4.47 4.26

NOTE:— *Acetaldehyde pressure, 13.5 cm. O_2/Ac ratio, 1.27.*

It will be seen that an increase in surface increases the rate of reaction, but that a maximum effect is apparently reached when the surface is about five times that of the reaction vessel. A further increase in surface has little effect on the reaction velocity, but it does have some effect on the form of the pressure-time curves. This effect can be seen from the affine curves in Fig. 6, and consists mainly of an accentuation of the induction period.

An increase in surface also causes a change in the maximum pressure decrease accompanying the reaction, as shown by Table XI.

It is therefore apparent that the secondary reactions which cause the ultimate pressure increase are much more pronounced with the larger surface. The effect of the surface will be referred to again in the next section.

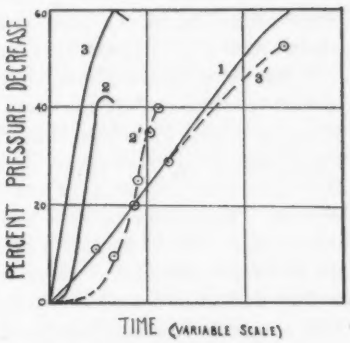

FIG. 6. *The effect of surface.*

Curve No.	*1*	*2*	*2'*	
Total surface / Original surface	*1*	*10*	*10*	*(affine)*
Curve No.		*3*	*3'*	
Total surface / Original surface		*5*	*5*	*(affine)*

TABLE XI

THE EFFECT OF SURFACE ON THE MAXIMUM PRESSURE DECREASE

Sample of glass, no.	Total surface / Original surface	Maximum pressure decrease, %	Sample of glass, no.	Total surface / Original surface	Maximum pressure decrease, %
1	5	61.5	2	10	45.9
3	10	48.9	none	1	77.0

NOTE:— *Temperature, 120° C. Acetaldehyde pressure, 13.5 cm. Oxygen/acetaldehyde ratio, 1.28.*

Discussion

In the first place, from the point of view of general theories of organic oxidation, it is apparent that the results obtained in this investigation are absolutely incompatible with any theory of the dehydrogenation type. The major step in the oxidation reaction is unquestionably the formation of a peroxide by the direct addition of oxygen. This is indicated both by the analytical results and by indirect considerations.

In virtually all gaseous oxidation reactions an induction period occurs. In order to explain the lag in reaching the maximum rate of pressure *increase*, it is customary to assume the intermediate formation of peroxides (7). In the present case an induction period also occurs; this is the reverse of the usual type, however, in that there is a delay in reaching the maximum rate of pressure *decrease*. Such an induction period obviously cannot be explained by the initial accumulation of peroxides. In a previous section it has been shown that the experimental data can be explained exactly on the assumption

that the reactions occurring during the induction period are not the same as those occurring in the later stages. While this is peculiar and no definite explanation can be given, it is possible to suggest how it may come about.

It has been shown above that an increase in surface tends to increase the rate of reaction and at the same time to accentuate the induction period. It seems probable therefore that the reactions occurring during the induction period are surface reactions. It will be shown later that the evidence points to the main reaction being of the chain type, with the chains initiated at the surface. The whole process will therefore be dependent on the condition of the surface. The adsorption of products from the reactions occurring during the induction period may therefore retard these processes and at the same time put the surface in a condition suitable for the development of the chain reaction. This suggested explanation is, of course, highly speculative. There is, however, no doubt that the reactions occurring during the induction period are quite distinct from, and cannot be the first step in, the subsequent reactions.

The kinetics of the oxidation of acetaldehyde have been previously discussed by Bodenstein (1). In Bodenstein's investigation a large excess of acetaldehyde was always used, so that the results are not entirely comparable with those reported here. The temperature coefficients of the two investigations, *viz.*, 10,000 and 8700 calories, are in as good agreement as could be expected. The general results are also in good agreement.

Bodenstein gives a complicated kinetic analysis of the reaction, but reports very little data to substantiate it, especially of an analytical kind. The main point in which the present investigation disagrees with Bodenstein's scheme is in connection with the induction period. This is explained in his paper as being due to the oxidation of impurities which act as inhibitors and are probably derived from stopcock grease. This assumption cannot possibly explain the present results. For example, in one case previously mentioned, at the end of the induction period 42% of oxygen had been used, and 18.2% of carbon dioxide formed. In addition, the total amount of condensable material had gone up to 123.5% of the initial quantity of acetaldehyde. These amounts of material cannot possibly have been derived from a trace of stopcock grease. The induction period was also found to be quite reproducible, which would certainly not be the case if it were due to the fortuitous presence of a trace of impurity.

Bodenstein's kinetic analysis of the oxidation of acetaldehyde will not be discussed further for several reasons. In the first place, it is rather complicated and has practically nothing to substantiate it. Secondly, there is no justification for dismissing the induction period as due merely to impurities. Finally, it has already been pointed out that the oxidation of acetaldehyde is attended by a number of complications. It is therefore a most unfortunate reaction to choose as a typical example on which to base a general theory of organic oxidations.

The oxidation of acetaldehyde is almost certainly a reaction of the chain type. It is true that an increase in surface increases the rate of the reaction,

but since the form of the pressure-time curves is altered, and especially since the effect of the surface reaches a maximum, the reaction is certainly not a simple heterogeneous one. The general trend of the results is analogous with those of other organic oxidation reactions of the chain type. Thus the order of the reaction is variable and not integral, and the rate is dependent on the aldehyde concentration and not on that of oxygen. The effect of increasing the surface indicates that the chains are initiated, at least largely, at the walls of the container. Since with increased surface a maximum rate is reached, the chains would also seem to be broken at the walls to a considerable extent.

The heat of activation of the reaction is much lower than that of a homogeneous bimolecular reaction occurring at the same rate at the same temperature. If, however, the primary step occurs at the walls of the vessel, this is not surprising since in the case of a heterogeneous reaction the heat of activation calculated from the temperature coefficient is only an apparent one, and will be lower than the true value by the heat of desorption of the reactants.

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THE VOLUME RELATIONS OF THE SYSTEM CELLULOSE AND WATER[1]

By Edgar Filby[2] and O. Maass[3]

Abstract

The absolute specific volume of cellulose has been determined by means of helium gas. A new experimental procedure has been developed, which, it is claimed, is more accurate than any previously used where a small quantity of porous material is involved. The method is extended to include the measurement of the volume of a system resulting from the adsorption of a vapor on a porous material, and applied in this instance to the system cellulose-water vapor. The specific volume of cellulose in helium was found to be 0.640 ± 0.001 cc. The volume of the system cellulose-water vapor was found to be much smaller than that given by the components cellulose and condensed water, as long as the amount of adsorbed water was below 4%. With more than 8% of adsorbed water the density of subsequently sorbed water was found to be the same as that of the normal liquid.

The hypothesis is put forward that the first water adsorbed on cellulose enters into a definite chemical combination, considerable contraction taking place, and that subsequently adsorbed water first causes an increase in cellulose surface, with further consequent contraction in the volume of the two components and then a filling up of the so-called capillary spaces in which the water maintains its liquid properties. The relation of this hypothesis to the hysteresis effect is indicated, the trend which further work should take is pointed out, and the interest of this problem from a practical point of view is discussed.

Introduction

The specific volume of cellulose has been under investigation since the time of Kopp (6) who used a "volumenometer" and air as a gaseous medium. The more recent and more trustworthy determinations of this property were made by the pycnometer method with various liquid media; Vignon (14), Guttmann (5), de Mosenthal (10), Cross and Bevan (2), Little (9), Richter (11) and Lewis (8). A good average value for the specific volume of cellulose in water was found to be 0.621. In 1927, Davidson (3) investigated a number of cotton samples by means of a gaseous method in which helium was used. These measurements showed cotton cellulose to have an absolute specific volume of about 0.640, which is the helium value obtained in the present work where measurements were also carried out in air and hydrogen.

Urquhart (12, 13) emphasized the idea that in nature cellulose is precipitated in the presence of water, which probably results in a certain retention of water molecules by the hydroxyl groups of the cellulose crystallites. On drying, the residual valencies which were formerly occupied by water molecules tend to satisfy each other, and the cellulose micellae join together to form fibres, while on being hydrated, the water enters the spaces between these micellae, satisfying their secondary valencies, and thus causing a swelling similar to that suffered by gelatine and other swelling gels.

If, while wetting a cellulose sample, the water-vapor pressure be measured

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at different moisture contents, and then the same experiments be made while drying the sample, it is found that a hysteresis phenomenon takes place, that is, the relative vapor pressure on the desorption isotherm is found to be lower than that on the adsorption isotherm for the same percentage moisture. Urquhart explained this phenomenon by assuming that dry cellulose, on being exposed to water, satisfies its residual valencies, causing those valencies which mutually attract each other on drying to be released, and the gel to disperse. Thus on hydrating, the active groups increase, while on drying they decrease, in this way giving the pronounced hysteresis effect. He also assumed that "during hydration only a definite fraction of those residual valencies which satisfied each other on drying and distorted the crystallites will be released". Thus it would follow that the drier the cellulose, the less water it will take up on hydration, which seems to assign to cellulose some non-swelling properties. It will be noticed on any adsorption or desorption isotherm of cellulose and water, that the vapor pressure, which, in the initial stages of adsorption rises very slowly, shows a sharp increase at approximately 3 to 4% of water and maintains this until the saturation point is nearly reached. This and other properties show some difference in behavior on the part of the water initially added and that subsequently added to a so-called dry sample of cellulose.

Among the other properties referred to above, the sorption of hydrogen chloride on cellulosic materials containing sorbed water has given evidence that two states of sorbed water exist, namely, chemically combined surface water, and water which is held by capillary attraction between adjacent cellulose micellae. The cellulose micella presents a surface of active groups to which water adds in a chemical sense and thus loses its properties, except in so far as a dissociation equilibrium exists. For purposes of designation the authors propose to call this "chemically bound water"; water subsequently sorbed will be held under a reduced pressure by virtue of the proximity of adjacent cellulose surfaces, a pseudo-curvature for this liquid water being calculable on the basis of the Gibb's equation. Water in this second state can be expected to retain approximately the properties of the normal liquid, though there may be slight modifications since the surfaces of chemically combined water will in turn impose some modifications on the adjacent molecular layer of water, and since the "capillary conditions" involving internal pressure as caused by the curvature of the free liquid water are bound to have some influence. The vapor pressure of such water will of course be dependent entirely on the curvature of the free liquid surface. It is to be expected that marked changes in properties of the chemically adsorbed water will result. Among others, an apparent change in density will take place, while water subsequently sorbed, namely liquid water, will show no such change.

The present investigation will show that the above hypothesis has been verified. It may be convenient however, at this point, to indicate a further development which it is hoped to establish. The sorption isotherm for water on cellulose is quite different from the desorption isotherm, the latter taking place at lower relative humidities, indicating that on desorption, larger amounts

of water are held at corresponding relative humidities. In the opinion of the authors, the adsorption of water is accompanied by an increase in cellulose surface which may be supposed to take place as follows: the cellulose micellae are held together in the first place by the mutual surface attraction of cellulose for cellulose; the water first adsorbed satisfies the free cellulose surface and subsequently added liquid water is able to compete with the cellulose for cellulose attraction, thus increasing the effective surface. The experiments cited above with regard to hydrogen chloride adsorption indicate that this phenomenon actually does take place. The water entering between the cellulose surfaces becomes chemically bound and therefore, on desorption, is not subject to evaporation at the same vapor pressure as that at which it was adsorbed. It is suggested therefore that there is a larger amount of chemically bound water present in cellulose during desorption than during adsorption. In this way an explanation for the phenomenon of hysteresis is obtained, and it is hoped that density measurements on cellulose subjected to desorption will prove the above theoretical considerations.

It was the object of this investigation, therefore, to design and construct an apparatus which could be used for the examination of the volume relation of a two-component system resulting from the adsorption of an easily liquefiable vapor on an adsorbent material, and to carry out these measurements in the case of the system cellulose-water.

In order to accomplish this a number of experimental difficulties required solution. It was necessary that the measurements take place in an isolated medium which does not adsorb on the cellulose surface to any appreciable extent, that the vapor pressure to which the sample is subjected remain constant throughout the determination, and that the moisture content of the sample be measured under these conditions.

Apparatus

The novelty of the experimental procedure employed consists of making use of a gas-expansion method. In this method a gas is allowed to expand either into, or from, a cell containing the system under investigation, the pressure changes recorded being used for the purpose of density calculations. Previous methods employing a gas for the density determination of a condensed system had as their basis the injection of a known volume of gas into the space containing the system. This is neither as accurate as the expansion method, nor does it admit of detecting and correcting for adsorption of the calibrating gas. For convenience the description of this new method is arranged under the following sections: expansion apparatus, apparatus for moisture determination, pressure control, accessories, arrangement of apparatus, and general manipulation.

The Expansion Apparatus

A. Two cells of about 10 cc. capacity were connected together by an oblique stopcock. One cell was then connected to the vacuum line through a stopcock, while the other volume was attached to a differential mercury manometer which was in turn attached to a constant-level manometer. This

second cell was also connected through a stopcock, phosphorus pentoxide tube and calcium chloride tube to the atmosphere. This apparatus is shown at *A* in Fig. 2.

B. Another apparatus was constructed similar to *A* (Fig. 1—*B*) except that cell V_1 was calibrated with mercury and a side tube was attached to V_2 through which the cellulose could be introduced. This side tube was part of a 5-cc. graduated pipette and was calibrated with mercury before being attached to the apparatus.

C. In order to obtain easier access to V_2, in which the cellulose was placed, a modified apparatus was built as shown in Fig. 1, *C*. In this the same general arrangement was followed, one calibrated cell being attached to the vacuum line and to a second cell, which in turn was connected to the gas line and differential manometer. The main difference was that the cell V_2 was supplied with a finely ground lid which fitted on to a glass rim after the manner of a desiccator top. The lid was held down by a very thin layer of vaseline and sealed with a small mercury reservoir.

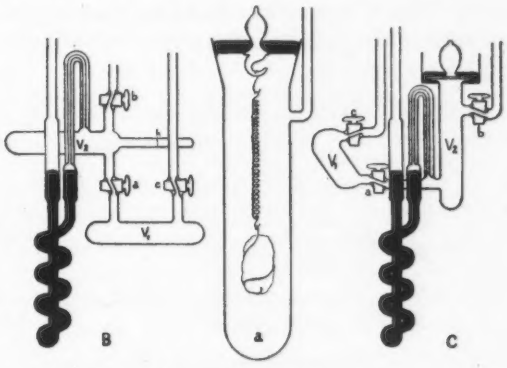

FIG. 1. *Detailed expansion apparatus B and C, and spiral balance a.*

The Apparatus for Moisture Determination

The moisture of the sample placed in V_2 was determined by measuring the elongation of a quartz spiral to which a sample of the same material was attached.

Two containers were made as shown in Fig. 1—*a*, and attached to the expansion apparatus as shown in Fig. 3. The containers were fitted with ground glass stoppers having a hook on the under side on which the quartz spiral could be hung. Measurements of the lengths of these spirals were made by means of a Becker cathetometer.

The quartz spirals were obtained by winding fibres on an automatic roller which operated like a screw, giving about 50 turns to the inch. The fibres were made from quartz rod which was heated in an oxy-acetylene flame and drawn out by attaching one end to a weight which was dropped about 12 ft. Fibres were made up to this length and found to be straight only when they were drawn out along a bench so that they were supported while cooling. The two spirals used in this work were tested for Hooke's law.

Many such spring balances have been set up and the sensitivity found to be proportional to the diameter of the helix and the number of turns per unit

length, and inversely proportional to the fourth power of the diameter of the fibre.
That is

$$S = K \frac{ND}{d^4},$$

where S is the sensitivity, N the number of turns per inch, D the diameter of the helix, d the diameter of the fibre and K some constant.

The Pressure Control

In order to keep the pressure equal on both sides of the differential manometer shown at A in Fig. 2, some device for introducing and extracting small

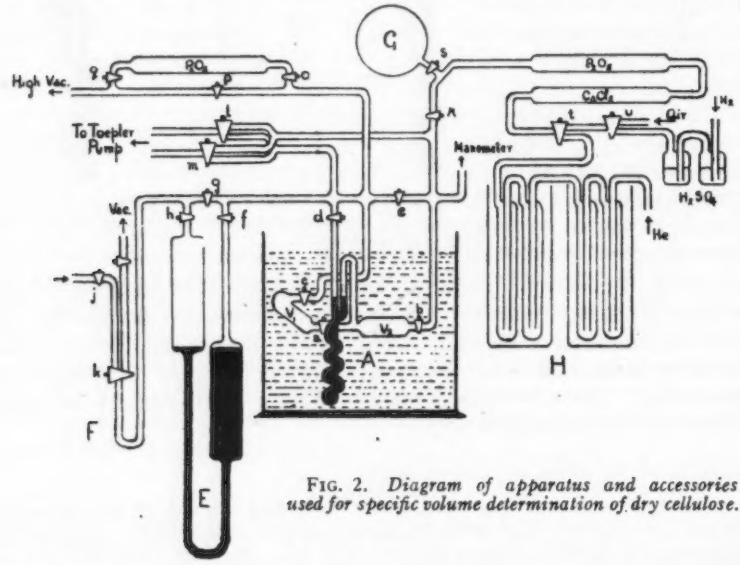

FIG. 2. *Diagram of apparatus and accessories used for specific volume determination of dry cellulose.*

quantities of gas from the left hand side of this manometer was needed. This was done through the arrangement of stopcocks shown at F in Fig. 2. As this apparatus is connected to the cylinder on the left hand side of U-tube E, which has a volume of about 800 cc., the introduction of a few cc. of gas at atmospheric pressure through stopcock k increases the pressure only very slightly. E is a U-tube both cylinders of which are of approximately the same volume, and was used to isolate the system from the pressure control when purified helium was being used as a gaseous medium.

Accessories

A Cenco Supervac consisting of a mercury-vapor pump backed by a Highvac oil pump was used in conjunction with an additional mercury-vapor pump. This combination gave a vacuum of less than 0.001 mm. of mercury in 10 min. when connected to a litre flask. A constant-level manometer was built and a thermometer to record temperature readings was attached to the mercury

column, behind which was placed a glass mirror scale. Low pressures were recorded on a McLeod gauge connected in series with this manometer. In order to purify helium four coconut-charcoal tubes, shown at H in Fig. 2, were made of Pyrex glass and hooked together in doublets so that they would fit into one-litre Dewar flasks. The tubes were filled with activated coconut-charcoal and connected to the apparatus and helium tank by de Khotinsky seals. To conserve the purified helium, a Toepler pump was built and attached to the line as shown in Fig. 2. Two automatic constant-temperature baths were set up to control the temperature of the expansion apparatus and the water bulb.

Arrangements of Apparatus

In Fig. 2 a diagrammatic sketch of the apparatus is shown. A is the thermostatically controlled expansion apparatus, the calibrated cell of which is connected through the stopcock c and the drying tube, to the Supervac pump. The cell V_2 has two outlets, one through the differential manometer and stopcock d to the constant-level manometer and pressure-control apparatus E and F, the other through the stopcock b to the manometer, which may be connected to the gas line through the stopcock r. The arrangement of stopcocks l and m which lead to the Toepler pump made it possible to pump gas to and from any part of the apparatus. The litre flask G was used for storing gas which was introduced into the apparatus through the drying tubes and the stopcocks t and u. A plug of cotton wool was used to filter out dust particles.

The apparatus for experiments using water vapor was set up as shown in Fig. 3, the water bulb being immersed in the usual constant-temperature water bath or Dewar flask containing an ice mixture, depending on the temperature required. This arrangement solved one of the main experimental difficulties, namely, the impregnation of the system to be examined for density with a definite known moisture content. The water vapor coming initially from n had to pass over the cotton on spiral S_1 then through the sample in cell V_2 and finally to the cotton on spiral S_2. When these two spirals S_1 and S_2 showed the same moisture content it indicated that the water-vapor pressure equilibrium had been established and that the cotton in cell V_2 had the same moisture content as that indicated by the two spirals. This point is emphasized to show the care taken to ensure that the moisture content of the cotton under investigation was definitely established.

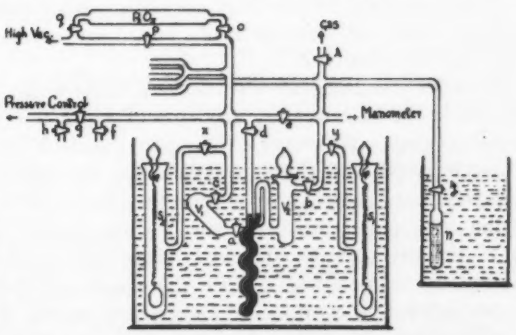

FIG. 3. *Diagram of device added to specific volume apparatus for determination of amount of adsorbed water.*

General Manipulation

Using expansion apparatus, A, and the set-up shown in Fig. 2, tests were made as to the reproducibility of results on expanding the gas in one cell at atmospheric pressure into the other cell when completely evacuated. This experiment was carried out as follows. The apparatus was opened to the atmosphere through the stopcocks r, t and u, thus obtaining the same pressure on both sides of the differential manometer, and the pressure was measured on the constant-level manometer at three different parts of the scale. A low-power microscope objective of about 60 cm. focal length was focused with the scale mark on the mercury meniscus at the right side of the differential manometer. Stopcocks a, b, d, and r were then closed and cell V_1 evacuated. When the pressure in V_1 as read on the McLeod gauge reached a low value of about .001 mm. Hg, stopcocks c and o were closed, e being left open. Next, the pressure on the manometer was adjusted to the pressure it was anticipated would be established in cells V_1 plus V_2 after the expansion had been made. When this was done, stopcock d was slowly opened, the mercury in the left side of the differential manometer gradually rising, until stopcock a was opened slightly, and the pressure on both sides of the manometer more nearly equalized. This operation took about one minute and great care had to be taken not to blow the mercury about. When stopcocks a and d were both open the pressure was adjusted through the control apparatus F until the mercury meniscus of the differential manometer was at the same scale division on the microscope as before, and thus the pressure on the constant-level manometer was the same as the pressure in cell V_2.

When purified helium was used stopcock g was closed, thus isolating the system from the apparatus F, and causing the pressure to be controlled through the U-tube E.

In order to make experiments using water vapor as well as helium, water was placed in the bulb n, and freed from air by alternately freezing and evacuating several times. When the entire apparatus had been evacuated with stopcocks r and z closed, and the bath containing the water bulb had been adjusted to the required temperature, stopcocks e and o were closed, a, b, c, d, x and y being left open. Stopcock z was then opened, and the entire system left until the two samples attached to the quartz spirals had acquired the same percentage of moisture. When this water-vapor equilibrium was reached the vapor pressure was measured on the manometer, the stopcocks a, b, c, x, y and z were closed, e was opened, and the vacuum pump connected to the system to extract the water vapor from the surrounding glass tubing. The system was then isolated from the pump and drying tubes, and helium was introduced through r until a definite pressure was reached, when r was closed. The gas was allowed to flow into cell V_1 by opening c very slightly, thus avoiding a loss of water vapor from the apparatus. The pressure of gas in V_1 was measured as usual on the constant-level manometer, and the expansion carried out as before.

Materials

Mercury

Commercial mercury was purified by pouring it through a nitric-acid tower three or four times, and by washing with water. This mercury was then distilled in a Pyrex apparatus under a partial vacuum and in the presence of a slow stream of air. The mercury was redistilled into the manometer and Toepler pump in order to prevent any contamination from dust particles in the air.

Cellulose

A sample of purified rag cellulose, which had been soda-boiled was obtained from the Eastman Kodak Company, and was kept in a conditioning jar. Samples for moisture determination were taken whenever a sample was to be used in the expansion apparatus described above.

Preliminary Work

The apparatus shown at *A* in Fig. 2 was used to test the expansion method which was to be used in the actual experimental work. The experiment described under the heading "Manipulation" was repeated many times and concordant results obtained.

A number of experiments were carried out using an apparatus of type B (Fig. 1). These determinations gave a value of 0.63 and 0.61 cc. for the specific volume of cellulose in air and hydrogen respectively. In both cases the pressure in the cell containing the cellulose was initially about 1 atm., and was reduced to about ½ atm. when the gas expansion was made.

Experimental Results

An apparatus of type *C* (Fig. 1) was found to be the most suitable for this work. With this, experiments were carried out in two ways: first, after the manner of those made in the preliminary work, where the pressure of gas on the cellulose was decreased from 1 to ½ atm.; second, by having cell V_2 and the hole in stopcock *b* initially at vacuum pressure and cell V_1 at atmospheric pressure, then carrying out the gaseous expansion so that the pressure on the cellulose increased from about 0.001 mm. of mercury to about ½ atm. The volume of cell V_1 was found before the apparatus was assembled, by filling with mercury and weighing at a given temperature (see Table I).

TABLE I

DETERMINATIONS OF THE VOLUME OF THE CELL V_1

Wt. of Mercury, gm.	Temp., °C.	Volume of V_1, cc.
137.133	28.0	10.1377
137.143	28.0	10.1384
137.143	28.0	10.1384
137.133	28.0	10.1377
Mean		10.1380

Calibration of V_2

After the apparatus had been assembled and attached in place of *A* in Fig. 2, 94.529 gm. of mercury was placed in the differential manometer and the volume of cell V_2 found by the expansion method previously described.

Letting p_1 be the initial pressure in cell V_1 in mm. of mercury, p_2 the initial pressure in cell V_2 in mm. of mercury, p_4 the final pressure in the combined volumes of V_1 and V_2 in mm. of mercury, T_1 the temperature of the constant-level manometer and P_1, P_2 and P_4 be the pressures corresponding to p_1, p_2 and p_4 after correcting to 0°C. for the expansion between mercury and glass, graphs being drawn for this from data taken from Landolt and Bornstein (7), then, if $P_1=0$, $V_2=\frac{V_1 P_4}{P_2-P_4}$ or, if $P_2=0$, $V_2=\frac{V_1(P_1-P_4)}{P_4}$ where V_1 and V_2 are the volumes of the respective cells in cc. Typical computations of V_2 are shown in Table II.

TABLE II
DETERMINATIONS OF THE VOLUME V_2

No.	p_2	p_4	T_1	P_2	P_4	V_2, cc.
1	728.3	419.8	28.5	724.8	417.7	13.788
2	713.1	410.9	24.3	710.1	409.1	13.780
3	724.4	417.5	25.4	721.3	415.7	13.788
4	730.6	420.9	24.8	727.5	419.1	13.776
Mean						13.783

The lid of cell V_2 was taken off and replaced and the volume of the cell redetermined. The results of this determination are given in Table III and show that, as far as these experiments are concerned, the removal and replacement of the lid did not change the volume of cell V_2.

TABLE III
DETERMINATIONS OF THE VOLUME OF V_2 AFTER REMOVAL AND REPLACEMENT OF THE LID

No.	p_2	p_4	T_1	P_2	P_4	V_2, cc.
1	756.1	435.8	24.4	753.0	433.9	13.783
2	759.8	437.8	24.0	756.7	436.0	13.780
3	754.2	434.6	25.6	750.9	432.7	13.784
4	752.2	432.5	25.9	748.8	431.5	13.789
5	745.7	429.7	28.1	742.1	427.6	13.784
Mean						13.784

The cell V_2 was packed with a sample of moist cellulose, dry weight 4.805 gm., which was then dried for a period of three or four days by evacuating in contact with phosphorus pentoxide.

Experiments using Helium as a Gaseous Medium

For a few experiments, helium from the tank was purified by passing over activated charcoal at the temperature of liquid air. When the four charcoal tubes shown in Fig. 2 had been sealed into the vacuum line they were heated with a smoky flame and the adsorbed gases drawn off by the pump. After heating for three- or four-hour periods on several occasions a fairly high vacuum was obtained while the tubes were hot, this of course gave a high vacuum as soon as the tubes were cooled by immersing them in liquid air.

The pressure of helium was allowed to build up very slowly by adjusting the two stopcocks between the charcoal tubes and the helium tank. Two determinations made with purified helium are shown in Table IV. In these, the cellulose was initially evacuated and cell V_1 filled with purified helium at about atmospheric pressure. The data from experiments made with the dry unpurified gas are shown in Tables V and VI. In view of the close agreement between the results with purified helium and with impure helium (Table IV Nos. 1 and 2, Table V Nos. 1 and 2) it was considered quite unnecessary to carry out the purification, and in all further experiments the unpurified gas was used. In the first determinations of Tables IV, V and VI, the final pressure, P_4, is recorded at various time intervals and the change in pressure after the initial expansion has taken place is given under the heading dp.

TABLE IV

RESULTS OBTAINED USING PURIFIED HELIUM AS A GASEOUS MEDIUM

No.	P_1	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	767.8	373.5	2				
		373.4	7				
		373.4	15				
		373.4	26	0.1	10.709	3.075	0.640
2	768.1	373.5	25	0.1	10.710	3.074	0.640

TABLE V

RESULTS OBTAINED WITH DRY UNPURIFIED HELIUM AS GASEOUS MEDIUM

No.	P_1	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	767.4	373.2	2				
		373.1	8				
		373.1	15				
		373.1	22	0.1	10.712	3.072	0.639
2	767.3	373.2	30	0.1	10.709	3.075	0.640
3	601.6	292.7	25	0.1	10.700	3.084	0.642
4	601.9	292.9	26	0.1	10.697	3.087	0.642
5	398.9	194.9	20	0.1	10.698	3.086	0.642
6	399.6	194.5	25	0.1	10.700	3.084	0.642

The above results show little variation of the specific volume of cellulose in helium over the pressure range investigated, also the almost instantaneous establishment of the equilibrium pressure. Both these facts indicate that helium is not adsorbed on cellulose, the small change of 0.1 mm. in the value

of dp being accounted for by diffusion of the gas into the inter-crystallite spaces.

TABLE VI

RESULTS OBTAINED USING DRY UNPURIFIED HELIUM AS GASEOUS MEDIUM

No.	P_2	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	746.7	383.2	2				
		383.3	8				
		383.3	15				
		383.3	20	0.1	10.694	3.090	0.643
2	747.2	383.7	25	0.1	10.695	3.089	0.643
3	597.5	306.8	30	0.1	10.703	3.081	0.641
4	598.3	307.3	20	0.0	10.703	3.081	0.641
5	497.9	255.7	31	0.1	10.704	3.080	0.641
6	497.5	255.6	32	0.1	10.706	3.078	0.640

Experiments using Water Sorbed on Cellulose

Several determinations were made using helium as a gaseous medium and varying the amount of water sorbed on the sample. At first little or no success was met with in an attempt to obtain consistent results. Considering case No. 1 in Table VII for example; after the entire system had been evacuated and isolated from the pump and drying tubes, the water bulb was opened and the vapor allowed to pass over the suspended samples. When both samples recorded the same percentage of moisture, cell V_2 was isolated from the rest of the system and the water vapor removed from cell V_1 and the surrounding tubing by the vacuum pump and drying tubes. As can be seen from Table VII a gradual increase in pressure occurred, following the expansion, probably due to water vapor evaporating from the cellulose surface. This made it impossible to state accurately the percentage of water vapor held by the sample in cell V_2.

The opposite effect is shown in case No. 2 in the same table. Here, after both suspended samples had acquired the same percentage of moisture, cells V_1 and V_2 were isolated and the water vapor allowed to remain in cell V_1. The drop in pressure observed after expansion was evidently due to a sorption of water vapor by the cellulose in V_2, since on filling V_1 with helium, some water vapor from the surrounding tubes would be swept into V_1, giving a greater water vapor pressure there than in V_2.

In order to avoid these sources of error, V_1, as well as V_2, was isolated from the system and water vapor withdrawn from the tubing through which the helium had to pass on its way to V_1. This tubing was then filled with helium above atmospheric pressure, and the gas allowed to flow into V_1 very slowly so that little or no water vapor could escape from the cell. In this way

the vapor pressure in V_1 was kept the same as in V_2 as evidenced by the fact that scarcely any change of pressure was noticeable after the expansion of gas into V_2 had taken place. This method was followed in all subsequent work.

The data of Table VII show that the specific volume of the system cellulose and water is not additive in the initial stages of water sorption. The onus of this is thrown entirely on the water in order to simplify the representation of results. In Table VII, P_1 and P_4 represent the true pressure of helium in the apparatus, *i.e.*, the manometer pressure corrected to 0° C., minus the water vapor pressure in V_1 and V_2, W_1 and W_2 represent the weight of water in grams sorbed per gram of cellulose on the quartz spirals S_1 and S_2 respectively, V_2 the calculated volume in cc. of the space around the cellulose in cell V_2, V the total volume of sorbed water found by subtracting V_2 for the dry sample from V_2 for the wet sample, v the volume of this water in cc. per gram of cellulose, and d the average density of the sorbed water assuming the volume of the cellulose to remain constant under these varying conditions.

TABLE VII

AVERAGE APPARENT DENSITY OF ADSORBED WATER VAPOR AT VARIOUS PERCENTAGES OF MOISTURE

No.	W_1	W_2	Vap. press.	P_1	P_4	Time, min.	V_2	V	v	d
1			4.6	761.1	374.0	3				
					374.4	17				
					374.9	40				
					375.4	80				
2			4.6	761.0	372.9	4				
					372.5	17				
					372.1	42				
					371.0	84				
3	0.0320	0.0318	4.6	755.5	368.6	4	10.644	0.058	0.0121	2.62
					368.6	25				
4	0.0325	0.0315	4.6	761.7	369.5	5	10.642	0.060	0.0125	2.56
					369.5	20				
5			0.0	753.9	370.3	20	10.502			
6			0.0	753.9	370.3	22	10.502			
7	0.0622	0.0618	10.5	726.3	358.9	5	10.377	0.125	0.0250	2.48
8	0.0634	0.0625	10.5	732.0	361.8	3	10.373	0.130	0.0260	2.42
9	0.1105	0.1074	15.4	725.9	363.0	3	10.140	0.362	0.0723	1.50
10	0.1590	0.1560	16.9	725.2	367.2	2	9.880	0.623	0.1240	1.27
11	0.1590	0.1570	16.9	725.2	367.3	4	9.879	0.624	0.1250	1.26

After experiment No. 4 in the above table the cell was repacked with cellulose because of an accident in which the mercury in the differential manometer was blown over into the cellulose. The mercury in the differential

manometer was not replaced by exactly the original weight and thus the volume of cell V_2 was slightly changed. However, this volume change is immaterial as the difference in volume of the space around the cellulose when wet and when dry is all that is required. The dry weight of this sample was 5.010 gm.

Discussion

The Specific Volume of Cellulose in Helium

The values given for the specific volume in purified helium (Table IV) show agreement with those in the unpurified gas (Table V), within the experimental error of about 0.005 cc. in the total volume of the sample, while the variation over a pressure range is sufficiently close to this error that no significance need be attached to it. It may be concluded that either the impurities in this sample of gas were too small to affect the volume of the cellulose to any measurable extent or that they were of a non-adsorbent nature. This is in agreement with the analysis of the gas which showed more than 95% of helium and less than 5% of hydrogen.

No effective adsorption of helium on the cellulose surface can take place as the specific volume remains practically constant over the pressure range, and the final pressure is almost instantly established. The value of 0.639 to 0.642 agrees very well with Davidson's (3) results of 0.638 to 0.642 for cotton boiled in soda and serves as a check upon the accuracy of the experiment.

The Density of Adsorbed Water Vapor

The data in Table VII which show the average density of the adsorbed water vapor at various percentages* of moisture, are plotted in Fig. 4 (top), and show an almost constant high density up to 4% of water, followed by a gradual drop along a line which appears to approach a density of one in an asymptotic manner at some high unattainable moisture content. Values may be obtained for the actual apparent density of water vapor being adsorbed at a particular percentage of moisture in the following manner. The volume of water adsorbed per gram of cellulose at a definite percentage of water vapor is subtracted from that adsorbed at another, so also are the corresponding weights of this water, and from these results the density of water being adsorbed at the mean value of the two percentages of moisture may be found. That is, if x be the total volume of water adsorbed on one gram of cellulose at a% of water and y be the volume corresponding to b%, then the density of the water being adsorbed at $\frac{a+b}{2}$ % of moisture is given by the equation

$$d = \frac{\frac{a}{100} - \frac{b}{100}}{x-y} \rho.$$

This calculation was carried out at various intervals in Fig. 4 (top) and also for those recorded in Table VII. The figures are shown in Table VIII and are plotted in Fig. 4, (bottom).

**Here and subsequently "percentage" refers to cellulose by dry weight, that is, grams of water per 100 gm. of cellulose.*

TABLE VIII

APPARENT DENSITY OF WATER VAPOR ADSORBED AT VARIOUS PERCENTAGES OF MOISTURE

From Table VI No.	Weight of water, $a/100-b/100$	Volume of water, $x-y$	$\frac{\%}{\frac{a+b}{2}}$	$\frac{d}{\frac{a-b}{100\,(x-y)}}$
(3)	0.0319−0.0000	0.0121−0.0000	1.6	2.62
(4)	0.0320−0.0000	0.0125−0.0000	1.6	2.57
	0.030−0.020	0.0115−0.0076	2.5	2.56
	0.040−0.030	0.0155−0.0115	3.5	2.50
(8)−(4)	0.063−0.032	0.0260−0.0125	4.7	2.30
	0.060−0.050	0.0242−0.0194	5.5	2.08
	0.070−0.060	0.0300−0.0240	6.5	1.67
	0.080−0.070	0.0376−0.0300	7.8	1.32
(9)−(8)	0.109−0.063	0.0723−0.0260	8.6	0.99
	0.150−0.130	0.1155−0.0965	14.00	1.05
(11)−(8)	0.158−0.063	0.1250−0.0260	11.05	0.96

The curve obtained is continuous, with a sharp drop in density from a constant value of approximately 2.5 below 3% of adsorbed water to a value of 1.0 above 8%.

When the whole field of work on the relation between water vapor and cellulose is considered, one continually finds abrupt changes in behavior between 3% and 5% moisture content. For instance, the slopes of all adsorption and desorption isotherms change sharply between these two percentages, and the relation between cellulose and hydrogen chloride (4) shows that below 4% of moisture, cotton adsorbs less hydrogen chloride than a dry sample under the same pressure, while above 4% of moisture the reverse is true.

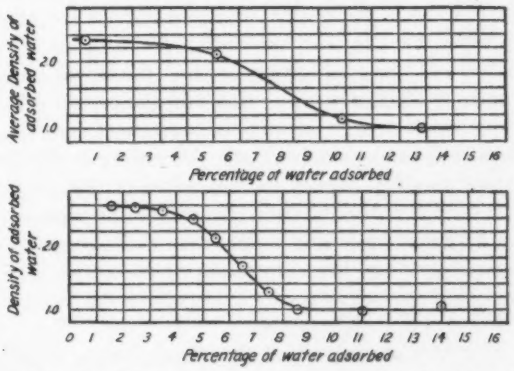
Average density of adsorbed water
2.0
1.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Percentage of water adsorbed
Density of adsorbed water
2.0
1.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Percentage of water adsorbed

FIG. 4. *Relations between apparent density of water and total amount adsorbed.*

In addition to the above, some conclusions drawn from crystal data and the density results may be of significance. If the average cellulose crystallite be considered to consist of 36 chains of 200 glucose units grouped together into a block 6 units by 6 units by 200, then there are 4872 units half exposed, which, upon the assumption of one water molecule for every completely exposed glucose unit, gives an adsorption of 2436 water molecules for 7200 glucose units, that is 3.7% by weight. Now the specific volume of cotton cellulose is

0.642 in helium and 0.621 in water*, a difference of 0.021 cc. in the volume of 1 gm. of cellulose in the two media. On a basis of this value of 3.7%, which is equivalent to 0.037 gm. of water per gram of cellulose, the volume of water which disappears per gram of water adsorbed, and takes up no space when cellulose is totally immersed in this liquid is $\frac{0.021}{0.037}=0.57$ cc. From this it is clear that 1 gm. of this kind of water occupies only $(1.00-0.57)=0.43$ cc., that is, its density is about 2.4. Considering the assumptions made, the value agrees very well with that found experimentally in this work. The ordinate of the density curve begins to decrease at about 3 to 4% of moisture, but the density of the water does not decrease immediately to the normal value. This may be due to more than one water molecule being adsorbed per glucose unit, this second molecule being less firmly held than the first. However, it is more likely that the true size of the micellae is less than that used in the above calculation, large parts of their surfaces being held together by secondary valencies which are replaced by water, once the initially free surface has been saturated. This apparent density curve is therefore in full agreement qualitatively, and to a certain extent quantitatively, with the hypothesis of water sorption put forward in the introduction.

Water in the high-density phase adsorbs until the exposed surface is saturated, *i.e.*, until about 3 to 4% of moisture has been adsorbed. Then, further water sorbs in the normal phase until, by its swelling action, it splits apart the micellae, creating new surfaces which can adsorb more water in the high density phase. If this supposition be true, the action would continue until the entire surface of all the micellae was exposed to water vapor, and from then on, the water would adsorb in the normal phase of density one, which takes place when about 8% has been adsorbed. According to this theory of water adsorption, a hysteresis effect should be noticeable in the density relation shown in Fig. 4, that is, if the density were determined at successive moisture contents as the cellulose was desorbing water, then the density corresponding to the moisture content between the extremities of the curve (3% and 8%) should be greater than that represented in Fig. 4, where the determinations were made as the cellulose was sorbing water.

It is very probable that the whole process of paper making is dependent on forces that come into play between cellulose fibres, the entanglement theory being relegated to second place in comparison with the theory of secondary valencies (compare Urquhart (12,13) Campbell (1)). Since the removal of water plays an important part in bringing into action these secondary valencies, any information concerning the binding force between cellulose and water is of considerable interest. Measurements are to be made on the heats of adsorption of water on cellulose at various concentrations, which, when combined with the density-volume changes investigated in this work, will make possible an estimation of the magnitude of this binding force.

**The cellulose is completely immersed in water as is the case when the pycnometer method of specific volume determination is used.*

In conclusion, it may be well to point out that the first addition of water to cellulose may be looked upon as a chemical combination, the volume of the water-cellulose compound formed being less than the sum of the volumes of the reactants, showing a definite contraction to have taken place. For convenience this effect has been tabulated in terms of the density of the adsorbed water.

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THE FREEZING OF WATER IN FISH MUSCLE AND IN GELATIN[1]

By J. H. Mennie[2]

Abstract

From the calorimetric measurements of Chipman and Langstroth (4) an estimate is made of the percentage of water frozen at temperatures down to −20° C. in samples of muscle from different species of fish. It is pointed out that if such measurements are to be used for the estimation of "bound" water, it must be known what portion of the water is kept from freezing by any inorganic salts that are present. Heat-capacity measurements on a 1.7% sodium chloride solution are given, and are shown to agree well with values calculated from available thermal data with the aid of the freezing-point curve. Heat-capacity curves for dry gelatin and 20, 40 and 66% gels are given. The unfrozen water in these gels at temperatures down to −20° C. is estimated and compared with results obtained by other methods.

Unfrozen Water in Fish Muscle

When animal or vegetable materials are preserved by freezing, only a part of the water which they contain is actually converted into ice. This is due partly to the lowering of the freezing point of the juice by the inorganic salts which it contains and partly, no doubt, to the "binding" of water by colloidal proteins. The question as to what proportion of the total water present is frozen at any given temperature is of interest in connection with the practical problems of freezing and cold storage.

Kallert (7) quotes some calorimetric measurements by Plank (12) from which he calculates that in meat 80% of the water is converted into ice at −10° C., 91% at −20° C., 99.9% at −50° C. and 100% only at −55° C. The calorimetric method was apparently used first by Müller-Thurgau (10, 11)† who made some measurements on apples and potatoes. Rubner (16)‡ and Thoenes (19) applied it to *Laminaria* and to various animal materials. Thoenes also made some measurements on gelatin and agar gels. The procedure described by Thoenes has been followed with slight modifications by Robinson (13, 14, 15) in a study of winter hardiness in certain classes of insects, and by St. John (18) in determining the unfrozen water in samples of egg white.

The total heat required to bring a sample of the material from some initial temperature T_1 below the freezing point to a final temperature T_2 above the freezing point, is measured in a calorimeter. From a knowledge of the specific heat of the material, the heat required simply to warm it through the temperature interval $T_2 - T_1$ is found. The difference between this and the observed value is attributed to the latent heat of fusion of the ice present, whence the weight of ice is obtained. Subtracting this from the total water content of the material gives the amount of water which remains unfrozen at the temperature T_1.

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Contribution from the laboratories of the Biological Board of Canada, Fisheries Experimental Station, Halifax, N.S.

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†*Quoted by Jones and Gortner (6).* ‡*Quoted by Thoenes (19).*

The equation given by Thoenes (19) and used with slight modifications by Robinson (13, 14, 15) and St. John (18) may be written

$$x = \frac{H - MS(T_2 - T_1)}{L_o + T_1(C_w - C_i)},$$

where x is the weight of water frozen at the temperature T_1 (T_1 being given its correct algebraic sign) in a sample of material of weight M. H is the heat absorbed as measured in the calorimeter, S is the specific heat of the material, L_0 is the latent heat of fusion of ice at 0° C. and C_w and C_i are the average specific heats of water and ice respectively between 0° and T_1.

This equation is discussed in detail in another paper (5) where the values 79.40 for L_0 and 0.55 for $(C_w - C_i)$ are recommended instead of 80 and 0.5, respectively, used by Thoenes. With the adoption of these constants, the equation has been used to calculate, from the measurements of Chipman and Langstroth (4), the percentage of water frozen in samples of muscle from different species of fish. The results are shown in Table I, in which the heat capacities given are average values read from the smooth curves. The equation is at best an approximation; the extrapolations on which it depends can be valid only for a comparatively short distance below the freezing point. Consequently, although Chipman and Langstroth carried their heat capacity measurements down to −70° C., no calculations have been made for temperatures below −20° C.

In the last column of Table I the amount of unfrozen water is shown, expressed in grams per gram of total solids. The samples of skate, herring and mackerel contained an appreciable quantity of fat (1.8, 13.3 and 25.2% res-

TABLE I

FROZEN AND UNFROZEN WATER IN FISH MUSCLE FROM HEAT CAPACITY MEASUREMENTS OF CHIPMAN AND LANGSTROTH (4)

Temp., °C.	Heat capacity, calories	Water frozen, %	Water unfrozen, gm.	Heat capacity, calories	Water frozen, %	Water unfrozen, gm.
Cod (80% *water*)				*Haddock* (80% *water*)		
−5	72.30	81.4	0.75	72.3	81.4	0.75
−10	80.75	91.3	0.35	79.8	89.6	0.41
−20	88.30	96.3	0.15	86.5	93.0	0.28
Burbot (83% *water*)				*Skate* (80% *water*)		
−5	75.6	83.6	0.80	60.0	61.4	1.70
−10	82.4	90.6	0.46	74.7	81.0	0.84
−20	98.8	93.7	0.30	84.2	88.8	0.50
Herring (65% *water*)				*Mackerel* (58% *water*)		
−5	67.5	90.5	0.29	54.4	78.6	0.72
−10	74.0	98.2	0.06	64.3	95.2	0.16
−20	80.0	99.9	0.00			

pectively) and a correspondingly lower proportion of water. In these cases the amount of fat was subtracted from the total solids and the figures given show the relation of unfrozen water to solids other than fat. The figures for cod, haddock and burbot are noticeably lower than those obtained for a 19.5% gelatin gel (Table V). Apparently there is less bound water in fish muscle than in a gelatin gel of similar water content. However, it must be added that the unfrozen water is a small difference between frozen and total water. Chipman and Langstroth give average values for the total water content and a small variation in it would mean a relatively large alteration in the figure for unfrozen water.

Most of the previous calorimetric measurements already mentioned were made with the object of estimating the amount of water bound by the colloidal material used. The unfrozen portion of the water has generally been assumed to be bound. In animal or vegetable tissues more or less inorganic matter is always present; fish muscle, for instance, contains 1 to 2% of salts, largely sodium chloride. In any salt solution the water will not all freeze until the temperature of the eutectic point is reached. Hence in a system which contains inorganic salts, at temperatures above the eutectic the unfrozen portion of the water will include the amount necessary to hold in solution at that temperature the salts which are present. The eutectic temperature of sodium chloride is −21.1° C. At a temperature even a fraction of a degree above this, the weight of water prevented from freezing by the salt is over three times the weight of the salt itself. The eutectic temperature of muscle juice is not known but is probably not above −20° C. (17). As most of the measurements made by the calorimetric method have been at temperatures not lower than −20°C., the effect of even small amounts of inorganic salts cannot be safely ignored.

Heat Measurements on Salt Solutions

Barnes and Maass (1) have made some heat capacity measurements on potassium chloride solutions and have shown that the heat capacity could be calculated with considerable accuracy from existing data on the specific heats of the components and the heat of solution. Their calculation was made for a solution of nearly the eutectic composition and at a temperature below the eutectic. It was thought worth while to make some measurements on a dilute salt solution and to compare the observed values with a heat capacity curve derived from available thermal data with the aid of the freezing-point curve.

A solution containing 1.7% of sodium chloride was used. The heat capacity measurements were made in an adiabatic calorimeter which had been constructed by Dr. H. Ritchie Chipman and used by Chipman and Langstroth (4). The calorimeter itself and the method of operation are described in their paper. The solution was placed in a cylindrical brass container which was closed by a screw cap. This was suspended by a thread and lowered into a brass cylinder, closed at one end, into which it fitted closely. The cylinder was immersed in the cooling bath which consisted of ice and brine for temperatures down to −10° C., and ether cooled with solid carbon dioxide for lower temperatures.

The bath was hand-regulated by the addition of ice or of carbon dioxide as required, and the temperature was read on a toluol thermometer. At least an hour was allowed for the solution to come to the temperature of the bath, then the container was drawn out by the thread and transferred as quickly as possible to the calorimeter. From the known heat capacity and the fall in temperature of the calorimeter the heat absorbed by container and contents was found. This was corrected in all cases to the same final temperature. The heat capacity of the container alone for the same temperature interval, found in exactly the same manner, was subtracted. Dividing by the weight of solution in the container then gave the heat capacity of the solution per gram. Table II shows the values obtained for the heat capacity per gram between various initial temperatures and a final temperature of 20° C. The weight of solution used was 10.892 gm.

TABLE II

HEAT CAPACITY PER GRAM OF A 1.7% SODIUM CHLORIDE SOLUTION BETWEEN VARIOUS INITIAL TEMPERATURES AND A FINAL TEMPERATURE OF 20° C.

Initial temp., °C.	Heat capacity, calories		Initial temp., °C.	Heat capacity, calories	
	Obs.	Calc.		Obs.	Calc.
0.0	19.1	19.5	−22.2	107.9	108.7
−1.5	54.2	50.0	−22.5	104.6*	104.8*
−1.5	61.5	50.0	−22.7	104.9*	104.9*
−2.0	63.0	61.5	−23.7	105.1*	105.6*
−5.7	86.4	88.1	−24.4	105.4*	106.0*
−10.5	95.1	95.3	−24.5	107.9	109.8
−15.4	99.9	99.9	−24.7	108.7	109.9
−17.3	101.4	101.4	−41.3	116.6	117.2
−19.7	103.1	103.0	−65.4	126.1	126.8
−21.5	107.7	108.3			

**Supercooled.*

The heat capacity was calculated in the manner suggested by Barnes and Maass (1). For temperatures below the eutectic the equation they give was used. This may be written:

$$H = aC_k(T_2 - T_1) + (1-a)(C_w T_2 - C_i T_1 + L_o) + aS,$$

where T_1 and T_2 are the initial and final temperatures, a the weight of salt per gram of solution, C_k the specific heat of the solid salt, C_i the average specific heat of ice between T_1 and 0° C., C_w the average specific heat of water between 0° C. and T_2, L_o the latent heat of fusion at 0° C. and S the heat of solution per gram of salt at T_2. The values for C_w, C_i and L_o were taken from the paper of Barnes and Maass (2) and those for C_k and S from the International Critical Tables.

When T_1 lies above the eutectic, the water is not all frozen and a slightly different equation must be used. The warming of the solution from T_1 to T_2 may be regarded as proceeding by way of the following steps: (a) The concentrated solution (salt plus unfrozen water) at T_1 is warmed to T_2. (b) The ice is warmed from T_1 to 0° C., melts at 0° C., and the resulting water is warmed

from 0° C. to T_2. (c) The concentrated solution is diluted at T_2 by the water from the melted ice. The total heat required is then given by:

$$H=(a+x)C_s(T_2-T_1)+(1-a-x)(C_wT_2-C_iT_1+L_0)+D.$$

Here x is the weight of water remaining unfrozen at T_1, which may be found from the freezing-point curve, and C_s is the specific heat of a salt solution containing a gm. of salt in x gm. of water. D is the heat of dilution at T_2 when enough water is added to $(a+x)$ gm. of this salt solution to make the total weight 1 gm. The freezing-point data and the values of C_s and D were taken from the International Critical Tables. The heat of dilution in no case exceeds 0.2 cal. and has been neglected in making the calculations.

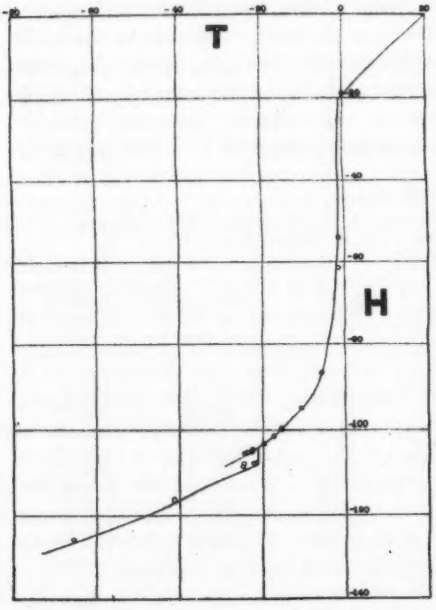

FIG. 1. *Heat capacity curve for 1.7% sodium chloride solution.*

From these equations a number of points were calculated and plotted, giving a smooth curve. The figures given as "calculated" in Table II were read from this curve. The calculated curve and the experimental points are shown in Fig. 1, which shows very clearly the excellent agreement between the observed and calculated results. Its most interesting feature is the evidence it exhibits of supercooling of the liquid or perhaps more correctly, supersaturation, below the eutectic point.

Briggs (3) has recently drawn attention to the necessity of considering the presence of inorganic salts in calculations of bound water. He gives the results of some calorimetric measurements on sodium chloride solutions from which he calculates the unfrozen water by means of Thoenes' equation. One of his solutions contained 1.7% of salt. For the sake of comparison his method

TABLE III

UNFROZEN WATER PER GRAM OF SALT IN A 1.7% SODIUM CHLORIDE SOLUTION

Author			Briggs		
Temp., °C.	Calc.	Obs.	Temp., °C.	Calc.	Obs.
−15.4	4.22	4.06	−15	4.50	4.99
−19.7	3.46	3.16	−20	3.46	3.24

of calculation has been applied to the figures at −15° and −20° C. taken from Table II. The result is shown in Table III. The "calculated" values were derived from the freezing point curve and the "observed" values from the heat-capacity measurements. The figures which Briggs actually gives apply to 1 gm. of solution and have been recalculated for 1 gm. of salt.

Heat Measurements on Gelatin

A number of measurements were made on gelatin gels of various concentrations. The gelatin used was Difco "Bacto-gelatin". By drying to constant weight at 110° C., it was found to have a moisture content of 14.7%; the ash content was 2.8%. Heat capacity curves were obtained for 19.5, 40.5 and 66.3% gels, and also for the dried gelatin.

The 19.5% gel was made up by weight, allowance being made for the water content of the gelatin, and was warmed on the water-bath until of uniform consistency. The liquid was then poured into a glass tube of slightly smaller internal diameter than the brass container. The tube was sealed and heated for 30 min. at 110° C., to sterilize the contents. When the gel had set, it was frozen quickly in carbon dioxide and ether. On freezing, the expansion of the gel burst the glass tube, whereupon the glass was removed and the solid cylinder of frozen gel dropped into the container and the cap screwed on. This procedure was adopted to avoid bursting the brass container on freezing the gel. The more concentrated gels did not expand so markedly and were made up directly in the container. A weighed amount of gelatin was placed in the container and the calculated amount of water added. The lid was then screwed on and the whole heated in the oven at 110° C. until the gel was of uniform consistency. In order to determine the period of heating necessary, similar amounts of gelatin and water were sealed in a glass tube and placed in the oven with the container. When the gel in the glass tube appeared to be uniform it was assumed that the same condition existed in the container.

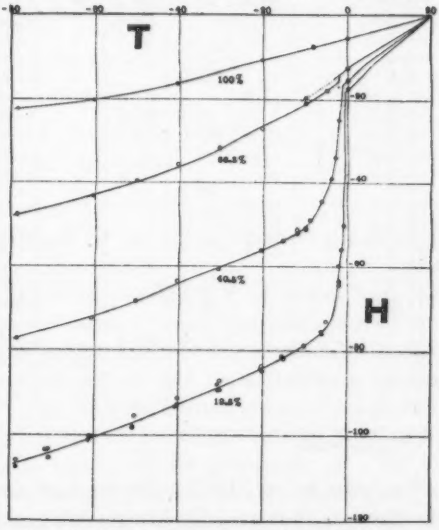

FIG. 2. *Heat capacity curve for gelatin gels.*

The first sample of 19.5% gel was left in a cold-storage room at about −19° C. between runs. With the succeeding samples (except the dry gelatin) supercooling was avoided by freezing the gel first in carbon dioxide and ether and then allowing it to warm up to the initial temperature in the constant-temperature bath. From this point the procedure was the same as already described. The results are shown in Table IV and in Fig. 2.

Two or three runs (indicated by an asterisk in Table IV) were made at temperatures a few degrees below zero with the container cooled directly to the initial temperature in the constant-temperature bath without the preliminary freezing mentioned above. In each case the points lie on, or very close to, the extension of the straight line drawn through the 0° C. point, showing supercooling to have occurred. To avoid confusion, only one of these points, *viz.*, that for the 66.3% gel, has been shown in Fig. 2. For the same reason, the point at −2° C. for the 66.3% gel is also omitted.

TABLE IV

HEAT CAPACITIES OF GELATIN GELS FROM VARIOUS INITIAL TEMPERATURES TO A FINAL TEMPERATURE OF 20 ° C.

Initial temp., °C.	Dry gelatin	66.3% Gel	40.5% Gel	19.5% Gel (1)	19.5% Gel (2)	Initial temp., °C	Dry gelatin	66.3% Gel	40.5% Gel	19.5% Gel (1)	19.5% Gel (2)
	Heat capacity						Heat capacity				
0.0	5.6	13.0	15.5	17.6		−20.4			55.9		
−1.0					50.2	−20.5				84.1	
−1.5					55.7	−30.2					89.2
−2.0		16.5	25.3	20.2*	59.8	−30.3					87.0
−2.0				63.7		−30.5		31.7			
−2.0				64.5		−30.6				87.9	
−3.0			33.7			−30.7				89.4	
−4.0			39.0			−40.2					91.3
−5.0		17.9			72.9	−40.2					92.8
−6.0			20.4*	75.6		−40.4			63.8		
−6.0			44.8	76.1		−40.5		35.4			
−8.0	7.7					−40.6	16.4				
−8.0	7.9					−40.7				93.2	
−9.0			49.0			−50.2		39.7			
−10.0		19.6*	50.7		77.2	−50.4			68.0		96.3
−10.0		20.9	50.8			−50.8				97.8	
−10.0		21.1	50.9			−50.9				98.2	
−10.2				79.0		−60.4		43.5			
−12.0			51.1			−60.6	20.2				100.3
−12.0			51.8			−60.8			72.2		
−15.1					81.7	−61.1				100.2	
−15.5			53.6	81.3		−61.3				101.1	
−20.2					84.5	−70.9					103.2
−20.3	11.2	27.6			83.4	−71.5				103.4	
						−78.5	22.4	47.9	77.5	107.4	105.9

*Supercooled.

Two samples of 19.5% gel were used, and in Fig. 2 the points obtained with the first are indicated by black circles and with the second by plain circles. Both were prepared at the same time, but one was stored for a month in a sealed glass tube before being frozen and inserted in the container. There is no evidence that this prolonged storage influenced the amount of water frozen, since both sets of measurements fall on the same curve. Repeated measurements at the same temperature showed satisfactory agreement, with no indication of an alteration in the amount of water frozen as a result of repeated freezing and thawing. When removed from the container the gels appeared clear and uniform and there was no sign of any separation of water. These

facts are in agreement with the observations of Jones and Gortner (6) made on the same material (Difco "Bacto-gelatin") by the dilatometer method. They found that repeated freezing and thawing or storage up to five days at room temperature did not alter the amount of water which would freeze.

They concluded from their results that all the water which could be frozen down to −50° C. was frozen at the initial freezing temperature (about −6° C.), if sufficient time was allowed for establishment of equilibrium. That is, when equilibrium was once established at about −6° C., no more ice was formed when the temperature was lowered further. On the other hand, Moran (8, 9) found by separating the ice which forms entirely on the exterior of a thin disk of gel when it is slowly frozen, that the unfrozen portion contained 54.3% of gelatin at −3° C. and the concentration increased to 65.5% (corresponding to 0.53 gm. water per gm. gelatin) at −19° C., after which further lowering of the temperature produced no further changes.

TABLE V

UNFROZEN WATER IN GELATIN GELS

Temp., °C.	19.5% Gel		40.5% Gel		66.3% Gel	
	Heat capacity, cal.	Unfrozen water, gm.	Heat capacity, cal.	Unfrozen water, gm.	Heat capacity, cal.	Unfrozen water, gm.
−3	69.0	0.92	34.0	0.96	16.4	0.48
−5	74.6	0.62	42.4	0.73	17.9	0.48
−10	78.8	0.51	50.4	0.57	21.0	0.48
−15	81.5	0.50	53.3	0.56	24.0	0.48
−20	83.8	0.50	55.6	0.58	26.9	0.49

In Table V is shown an estimate, made by means of Thoenes' equation in the form already used, of the water which remains unfrozen at temperatures down to −20° C. The heat capacities shown in Table V are taken from the curves of Fig. 2. The unfrozen water is expressed as grams per gram of dry gelatin. There is unmistakably a gradual freezing out of water down to some temperature between −10° and −15° C. There can be no question here of failure to establish equilibrium, since the gels were immersed in carbon dioxide and ether for at least half an hour before being brought to the initial temperature for each run. The amount of water remaining unfrozen at −20° C. is distinctly less than that found by Jones and Gortner (6), namely: 0.70, 1.05, 1.90 and 4.675 gm. in 32, 16, 8 and 2% gels respectively. The results in Table V are in quite good agreement with Moran's work (8).

On the basis of Moran's results (8) it was expected that the 66.3% gel might not freeze at all. Some of the water in it did freeze, however, as the break in the curve (Fig. 2) shows. Apparently this freezing all took place a little below 0° C. and further lowering of the temperature seems to have caused no increase in the amount frozen. It is possible that the somewhat prolonged heating used in the preparation of this gel resulted in some hydrolysis of the gelatin.

The general appearance of the curves in Fig. 2 is in agreement with Moran's (9) conclusion that there is no further freezing out of water at temperatures below $-20°$ C. Calculations made with Thoenes' equation from the measurements at the lower temperatures are useless and other methods of calculation require a knowledge of the specific heats of the dry gelatin and of the unfrozen water (5). It was expected that the latter could be derived from further measurements on a gel of 66% or higher concentration, but the work was interrupted before this had been done. The measurements are now being repeated with improved equipment and technique, and using ash-free gelatin.

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HEAT CAPACITY MEASUREMENTS ON GELATIN GELS[1]. I.

By W. F. Hampton[2] and J. H. Mennie[3]

Abstract

Heat capacity measurements using an adiabatic calorimeter have been made on a 24% gel, on dry gelatin and on gelatin containing 12.5% water. The calculation of bound water from calorimetric measurements is discussed, and the limitations of the method used by previous workers indicated. A new equation is derived and an estimate of bound water in the 24% gel given. Further work is in progress.

Introduction

Although there is ample evidence for the existence in lyophilic colloid systems of what is called "bound" water, very little is definitely known concerning the nature and intensity of the forces by which the water is held. In fact, there is no really adequate definition of bound water. When, for instance, a gel is brought to a low temperature, only part of the water present is found to freeze. The unfrozen portion is generally assumed to be "bound" by the colloid. Various other methods of estimating bound water are similarly based on the assumption that the bound water is that portion of the water content of the system which fails to display the normal behavior of ordinary "free" water under the particular experimental conditions used. The result is, according to Briggs (5), that "there are almost as many definitions of bound water as there are methods for determining it."

In a comprehensive review of the problem of water-binding in colloids, Kuhn (9) enumerates various ways in which water might be pictured as held by the colloid. These are not necessarily all distinguishable one from another but represent rather binding forces of varying intensity. He regards all the water contained in a gel as "bound" by the colloid and considers that the various physico-chemical methods which have been employed for the determination of bound water give only an estimate of the intensity of the binding forces.

This is essentially the same as the view advanced by Briggs (5) on thermodynamic grounds. He considers that the amount of water associated with or bound by a given quantity of colloid is not a constant but is determined by the activity of the water. Hence the amount of bound water found experimentally will depend on the conditions of temperature, etc., under which the experiment was made. He has attempted to correlate measurements made by different methods by taking into account the activity of the water in the system under the conditions of the measurement. He found the relation of activity to bound water content obtained by Newton and Gortner's (14) cryoscopic method for gum arabic, to be in good agreement with values derived from the vapor-pressure curve. Estimates of bound water in gelatin, agar and several other

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gels, however, made by Robinson's (18) calorimetric procedure, gave considerably higher values for the same activity than those indicated by vapor pressure measurements.

According to Briggs, measurements made on frozen gels should yield a result for bound (unfrozen) water which depends on the temperature of the frozen material but which is independent of the amount of water originally present in the gel. Jones and Gortner (8) on the other hand found, in a series of measurements made on gelatin gels by the dilatometer method, that if sufficient time was allowed for equilibrium to be established at the temperature where freezing first began, about −6° C., no more ice appeared to be formed even when the temperature was brought as low as −50° C. They also found that the amount of water bound by the gelatin was dependent on the concentration of the gel. The relation between bound water and gel concentration was a logarithmic one from which they infer that the binding of water by gelatin is an adsorption process. To account for the fact that the amount of water remaining unfrozen was apparently independent of the temperature, they suggest that there is an increase of "adsorption pressure" as the temperature is lowered which is just sufficient to counterbalance the decrease in the vapor pressure of the ice. This is equivalent to saying that the activity of a fixed concentration of bound water varies with temperature in exactly the same way as the activity of ice.

Moran (12) observed that when gelatin gels of 12 to 40% concentration, in the form of thin disks, were frozen slowly at −3° C., ice formation occurred entirely on the surface. The outer layer of ice could be removed from the core of partly dehydrated gel and the concentration of the latter determined. On lowering the temperature further, water passed from the unfrozen core into the outer layer of ice and the concentration of the unfrozen gel increased from 54.3% at −3° C. to a maximum of 65.5% at −19° C. corresponding to 0.53 gm. water per gram of gelatin. Moran called this the bound water and designated the water which separated between −3° and −19° C., "interstitial" or "capillary" water. Later measurements by the same method (13) gave closely concordant results with three different samples of ash-free gelatin, for gels of various concentrations within the limits 12 to 40%, and for widely different rates of freezing. Bound water according to Moran is apparently independent both of gel concentration (within the limits stated) and of temperature (below −19° C.). Even three days' immersion in liquid air caused no further separation of ice from a sample which had been first frozen at −20° C. (12).

There are thus considerable discrepancies in the results reported for gelatin gels even when only those methods which depend on freezing the gel are considered.

Experimental

Method

The present paper gives an account of the first stages of an investigation employing the calorimetric procedure which was used by Mennie (11). Most

of the previous measurements made by this method were on such complex systems as animal tissue, etc. As Mennie has pointed out, these are of little value in estimating the water bound by the colloids present since at the temperature used (−20° C.) it is improbable that the eutectic point of the muscle juice had been reached (20) and some of the water would be prevented from freezing by the inorganic salts present. Briggs (5) has also drawn attention to this fact.

In the present work "ash-free" isoelectric gelatin supplied by the Eastman Kodak Co. (Lot No. 48) was used. The ash content actually determined was 0.04% and the moisture content was about 12%. The pH was not determined but was stated to be 4.85.

The method involves the determination of the heat required to warm a sample of gel from various initial temperatures up to the temperature of the calorimeter. All observations were corrected to the same final temperature, 25° C., and a heat capacity curve constructed. A whole series of measurements were made on a single sample of gel which had been sealed in a container. To ensure uniform freezing conditions and make certain that equilibrium had been established, the gel was rapidly frozen in solid carbon dioxide and ether before each determination, and was then allowed to warm up to the desired initial temperature in a bath in which it was kept at constant temperature for 1 to 1½ hr. Mennie (11) had found no evidence that repeated freezing and thawing produced any permanent change in the gel which would prevent repetition of results with the same sample. This was confirmed with a freshly prepared gel by making three consecutive runs at −78.5° C. The values obtained for the total heat capacity of container and gel were 2648.4, 2653.1 and 2644.8 cal. At the end of a long series of measurements on this sample a fourth run was made at −78.5° C. giving a value of 2635.9 cal. This is somewhat low compared with the earlier figures. However, as Barnes and Maass (4) point out, the difficulties inherent in the method of transferring the container from bath to calorimeter tend to give low results, especially at low initial temperatures. If the difference were actually due to a change in the gel it would indicate a decrease in the amount of free water, a sufficiently improbable result which is not supported by any of the other measurements on the same gel.

Calorimeter

The adiabatic calorimeter used was that designed by Barnes and Maass (3) and used by them to measure the heat capacity of ice (4). They have given a complete description of the apparatus and the method of operation. It is unique in that it employs a radiation thermel to detect differences in temperature between the inner and outer baths. This eliminates the difficulty of accounting for the heat capacity of the thermel which, in the ordinary form of adiabatic calorimeter, is immersed directly in the inner bath. It also permits of closing off the water in the inner bath from contact with the outside air, thus eliminating errors due to evaporation, etc.

The apparatus is made very sensitive to small temperature differences

between the inner and outer baths by placing the galvanometer scale about three metres away from the mirror. A deflection of 1 cm. corresponds to 0.003° C. Since a shift of 0.5 mm. on the scale can be noticed, a temperature difference of 0.00015° C. can be detected. With careful adjustment the spot of light can be kept within 1 mm. of zero on the scale so the two baths can be maintained at the same temperature within 0.0003° C.

A Beckmann thermometer was used to obtain the temperature of the outer bath of the calorimeter. It had been calibrated by the U.S. Bureau of Standards and all readings made were corrected according to the certificate supplied. The thermometer was standardized by comparing it with a standard thermometer at nine different points. Readings on the Beckmann were plotted against readings on the standard, and the straight line obtained was extrapolated to zero on the Beckmann. In this way the Beckmann zero was found to correspond to 23.65° C.

A constant-temperature bath was used to bring the sample to a known initial temperature before introduction into the calorimeter. For 0° C. a water-ice mixture and for −78.5° C. solid carbon dioxide moistened with ether was used. For temperatures between these points a large Dewar flask, filled with ether and stirred with a current of dry air, was used. The ether was brought to the required temperature by dropping in lumps of solid carbon dioxide, and the bath could be maintained at the desired temperature within 0.1° C. by the addition of small lumps of solid carbon dioxide at regular intervals.

The temperature of the bath was obtained by means of a platinum resistance thermometer which was read by the usual Wheatstone bridge arrangement using a plug-type resistance box in series with a calibrated mercury resistance of the type designed by Maass and Mennie (10), and the same galvanometer used in the thermel circuit. The platinum thermometer was calibrated by determining its resistance in melting ice, in solid carbon dioxide and in liquid air. The composition of the liquid air was determined by a gas analysis using pyrogallol as the reagent, and the corresponding temperature obtained from Claude (6). As the value for the normal boiling point of oxygen given by Claude is −182.0° C. while the International Critical Tables give −183.00° C. as the best value, 1° C. was subtracted from the temperature read from the composition. The barometric pressure was neglected as a change of 10 mm. affects the temperature only by about 0.01° C. The carbon dioxide temperature, however, was corrected for barometric pressure by Henning's formula (7).

As a check on the performance and accuracy of the apparatus, a test run on ice was made with the same platinum container which had been used by Barnes and Maass (4). The heat capacity per gram of ice between −78.5° and 25° C. was found to be 138.1 cal. as compared with 138.2 cal. obtained by Barnes and Maass.

Material

The ash-free Kodak gelatin used was in the form of sheets. The rate at which this absorbed water when immersed in it was determined. A weighed

strip of gelatin about 1 by 3 in. was immersed in water at room temperature for a definite time then quickly removed, blotted between two strips of filter paper and weighed in a covered petri dish. For gels of concentration between about 20 and 60% the desired amount of water can be introduced quite readily by immersing the gelatin in water for the predetermined time.

An amount of gelatin which would give about 20 gm. of a 24% gel was immersed in water for 10 min. It was then removed to a test tube and melted to a sol, being kept at 50° C. for 30 min., then poured into the container. At the same time duplicate portions were poured into a petri dish, weighed quickly and then dried to constant weight in an oven at 105° C. This required about 48 hr. The mean value of the duplicate determinations was 24.0% of gelatin. This was taken as the concentration of the gel.

Container for Gels

The construction of the container for the gel is shown in Fig. 1 (not drawn to scale). It was made entirely of monel metal except for a thin lead washer under the cover. Capillary spaces around the screws into which water might creep were avoided by countersinking the holes and turning the heads of the screws so that the bevelled edge fitted very exactly into the countersunk hole. The washer was of practically pure lead and was quite soft so that when the cover was screwed down firmly the joint was completely filled. The container was found to hold ether very well under a vacuum.

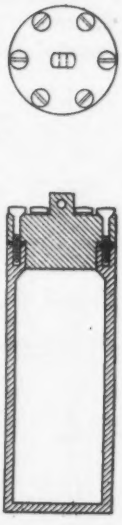

FIG. 1. *Diagram of container used for introducing gels into calorimeter.*

Two points had to be considered in filling the container. In the first place, sufficient space must be allowed for the expansion which occurs on freezing, to avoid bursting the container. In the second place, the gel should make as good contact as possible with the walls of the container in order to facilitate heat transfer and allow of equilibrium being established in the calorimeter as rapidly as possible. These requirements were met in the following manner. The sol which had been heated at 50° C. was poured into the container until the latter was about three-quarters filled. Immediately, the cover was screwed on as tightly as possible and the container rotated in a horizontal position at about 300 r.p.m. until the gel had set. Preliminary experiments showed that this caused the gel to set around the sides of the container, thus bringing into play as much surface as possible to aid in the transfer of heat, and also leaving a cylindrical space in the centre of the gel which permitted expansion on freezing. When the gel had cooled the whole was weighed. The container had already been weighed empty so the weight of the gel was determined.

The container was suspended from a cork by a silk thread of appropriate length and placed in a thoroughly dried copper tube closed at one end. The cork was sealed securely in the tube by means of adhesive tape to prevent moisture or ether creeping in. A piece of cotton wool was also introduced into

the tube above the container as an additional precaution. Between experiments the container was always kept in a desiccator over calcium chloride to ensure that it remained dry on the outside.

The copper tube enclosing the container was placed in a carbon dioxide-ether mixture for 10 min. to freeze the gel and was then transferred to the constant-temperature bath for 1 to 1½ hr. The container was then rapidly transferred to the calorimeter and the fall in temperature observed as described by Barnes and Maass (3). At the completion of the series of runs, the gel was removed from the container and its concentration checked by drying to constant weight. The result found was 23.7%.

Results

The results obtained for the total heat capacity of the 24% gel are shown in Table I. Table II shows the heat capacity of the empty container found in exactly the same way. The heat capacity of the dry gelatin was also deter-

TABLE I

TOTAL HEAT CAPACITY OF CONTAINER AND 24% GEL

Run No.	Initial temp., °C.	Heat capacity, observed, cal.	Heat capacity, average, cal.	Run No.	Initial temp., °C.	Heat capacity, observed, cal.	Heat capacity, average, cal.
	25.0		0.0	4	−38.3	1941.2	1941.5
14	0.0	606.1	607.0	6		1941.7	
15		607.8		11	−44.5	2061.1	2062.7
27	−0.6*	1008.1		12		2064.3	
28		839.3		9	−54.2	2237.1	2234.2
23	−0.8	1084.9	1087.4	10		2231.3	
24		1089.9		7	−63.8	2393.7	2397.5
20	−1.3	1128.3	1129.1	8		2401.8	
25		1129.9		1	−78.5	2648.4	2645.6
18	−5.3	1291.3	1293.6	2		2653.1	
22		1295.9		3		2644.8	
17	−15.2	1515.4	1516.7	29		2635.9	
21		1517.9					
13	−27.9	1756.3	1760.0				
16		1763.7					

**Temperature of freezing point.*

TABLE II

HEAT CAPACITY OF CONTAINER ALONE

Initial temp., °C.	25.0	0.0	−26.7	−57.8	−78.5
Heat capacity, cal.	0.0	−335.6	−686.4	−1077.9	−1339.5

TABLE III

TOTAL HEAT CAPACITY OF CONTAINER AND DRY GELATIN

Initial temp., °C.	25.0	0.0	−20.2	−34.9	−56.2	−78.5
Heat capacity, cal.	0.0	−389.5	−687.0	−909.4	−1207.2	−1523.8

TABLE IV

TOTAL HEAT CAPACITY OF CONTAINER AND 87.5% GELATIN

Initial temp., °C.	25.0	0.0	−29.2	−52.4	−78.5
Heat capacity, cal.	0.0	−414.4	−900.1	−1238.6	−1613.6

TABLE V

HEAT CAPACITY PER GRAM OF GELATIN GELS

Initial temp., °C.	Dry gelatin	87.5% Gel	24% Gel	Initial temp., °C.	Dry gelatin	87.5% Gel	24% Gel
	Heat capacity in calories				Heat capacity in calories		
25.0	0.00	0.00	0.00	−5.0			−71.88
0.0	−7.18	−8.21	−22.09	−10.0			−76.38
−0.6			−40.40	−20.0	−11.36	−14.78	−82.50
−0.6			−54.14	−40.0	−15.67	−20.32	−91.49
−1.0			−61.54	−60.0	−20.76	−24.64	−99.84
−3.0			−68.54	−78.5	−24.02	−27.72	−106.30

mined. A sample was cut up into small pieces and dried in an oven at 105°C. for 24 hr. and then to constant weight in a vacuum desiccator over phosphorus pentoxide for about two weeks. The dry material was then quickly packed in the container and the latter placed in an oven at 105°C. for about six hours before screwing on the lid. The heat capacities obtained are shown in Table III. A sample of the original air-dry material was also cut into small pieces and packed in the container. The moisture content was re-determined at the same time and found to be 12.5%, corresponding to 87.5% of gelatin. The heat capacities are shown in Table IV.

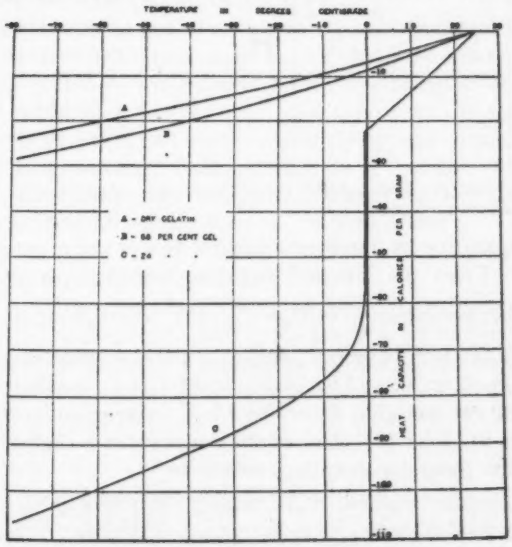

FIG. 2. *Heat capacity curves for gelatin.*

The data of Tables I to IV were plotted on a large scale and smooth curves drawn to fit them. From these curves the values for the heat capacities of the samples at various temperatures were obtained by subtracting the value read from the curve for the empty container at the same temperature. Dividing the result by the weight

of the sample gave the heat capacity per gram between various initial temperatures and the final temperature of 25°C. These values are shown in Table V and plotted in Fig. 2.

The Calculation of Bound Water

Several other workers (15, 16, 17, 18, 21, 22) have made use of the calorimetric method. Thoenes (22) has given an equation for calculating bound water from calorimetric measurements which may be written:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5T_1} \tag{1}$$

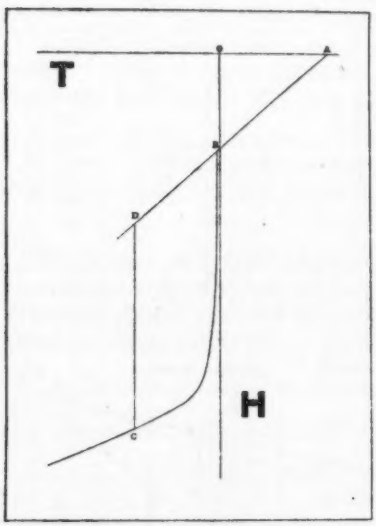

FIG. 3. *Heat capacity curve illustrating Thoenes' equation.*

where x is the weight of water frozen at T_1, H is the heat absorbed when the sample is warmed from T_1 to T_2, M is the weight of the sample and S its specific heat. The meaning of this equation may be discussed most conveniently by reference to Fig. 3. ABC is a typical heat capacity curve similar to that for the 24% gel shown in Fig. 2. The slope of AB gives the heat capacity per degree Centigrade (the quantity MS) of the unfrozen material. Extrapolation of AB to the temperature T_1 gives the point D. The heat capacity corresponding to D is evidently $MS(T_2 - T_1)$. The length of the line CD then represents $H - MS(T_2 - T_1)$. This is the heat liberated if the frozen portion of the water is melted isothermally at T_1. The weight of water frozen is obtained by dividing this quantity by the latent heat of fusion per gram *at the temperature* T_1.

From the Kirchoff equation for the rate of change of latent heat with temperature, $\frac{dL}{dt} = (C_w - C_i)$, the latent heat at T_1, $L_{T_1} = L_o + T_1 \frac{dL}{dt} = L_o + T_1(C_w - C_i)$ where C_w and C_i are the average specific heats between 0°C. and T_1, of water and ice respectively. If C_w is taken as 1 and C_i as 0.5 and L_o as 80 cal. per gm., $L_{T_1} = 80 + 0.5\ T_1$, corresponding to Thoenes' equation.

St. John (21) derives the equation in a slightly modified form which, using the foregoing notation, would be:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5(T_2 - T_o)} \tag{2}$$

where T_o is the observed freezing point of the material. From the above discussion it is evident that this modification is incorrect. The actual error in St. John's derivation was the use of 80 cal. for the heat of fusion *at* T_o.

If L_{T_0} is inserted in place of 80, the equation becomes identical with that of Thoenes.

The equation is an approximation at best. The specific heats of water and of ice are not independent of temperature. The specific heat curve of water is known only to −5°C. (1, 2) and it is questionable how far below that temperature it may legitimately be extrapolated. Extrapolation to −20°C. gives a mean value of 1.03 between 0° and −20°C. Taking the recent accurately determined values of Barnes and Maass (4) for the latent heat and specific heat of ice, the equation becomes:

$$x = \frac{H - MS(T_2 - T_1)}{79.40 + 0.55T_1} \tag{3}$$

The numerator still depends on the extrapolation of the line AB (Fig. 3) *i.e.*, on assuming a constant specific heat continuing far into the metastable or unstable region below the freezing point. From the appearance of curves A and B (Fig. 2) it seems probable that the extrapolated line should bend slightly toward the temperature axis. If so, the true value of CD would be slightly greater. Hence the equation probably gives values for the water frozen which are a little low. Since the bound water is usually quite a small difference between the total water content and the portion estimated to be frozen, this equation will give values for bound water which are likely to be high. In any case the extrapolations on which it depends, even if approximately valid as low as −20°C., the initial temperature used by Thoenes, can scarcely be relied on at temperatures any lower.

It is necessary then to find some other basis for calculation of bound water, which may be used over the whole temperature range covered by these experiments. When a gel at a temperature T_1 is warmed to T_2, the free water which is frozen at T_1 is melted and reabsorbed into the gel. The total heat change involved in this process is independent of the path by which the process occurs. It will be the sum of the heat effects which accompany any set of intermediate steps that may be assumed. The mechanism implied in Thoenes' equation is the isothermal melting of the ice and reabsorption of the free water at T_1 followed by warming of the gel to T_2. The possibility of a heat effect accompanying the reabsorption of the water is disregarded.

The gel might be assumed to be warmed to 0°C. without melting, the frozen portion of the water to be melted isothermally at 0°C., the whole to be warmed to T_2 and the free water reabsorbed isothermally at T_2. The heat capacity, H, of 1 gm. of gel between T_1 and T_2 would then be the sum of the heat effects involved in the individual steps, or:

$$H = aH_g + xH_x + (1 - a - x)H_i - \lambda. \tag{4}$$

Here H_g is the heat capacity per gram of dry gelatin, H_x is the heat capacity per gram of bound water, and H_i the heat capacity per gram of ice (including latent heat of fusion), all between T_1 and T_2. Total heat capacities rather than specific heats are used in this equation because they are the quantities

actually measured experimentally. In Equation (4), a is the weight of dry gelatin, x the weight of bound water, and λ the heat liberated when $(1-a-x)$ gm. of water are absorbed by a gel which already contains a gm. of dry gelatin and x gm. of water. The value of λ will depend on the value of x. According to Rosenbohm (19) the heat evolved when dry gelatin takes up water is nearly proportional to the amount of water up to about 0.25 to 0.3 gm. water per gm. of dry gelatin. At this point the curve bends rather sharply and the heat change accompanying further imbibition of water is small. Rosenbohm estimates that the entire heat of swelling is liberated when 0.5 gm. water has been taken up per gm. of dry gelatin. Moran (12) estimates the bound water to be 0.53 gm. per gram dry gelatin and a similar figure was obtained by Mennie (11) from earlier calorimetric measurements, using Thoenes' equation. If these estimates are correct, λ in the above equation should be zero or negligible.

Omitting λ, the amount of bound water is then given by

$$x = \frac{aH_g + (1-a)H_i - H_{obs.}}{H_i - H_x} \quad . \tag{5}$$

H_g, the heat capacity of the dry gelatin, is obtainable from the data of Table V. Values of H_i are given by Barnes and Maass (4). It is obvious that the specific heat of bound water cannot be the same as that of free water. A value must be found for H_x. An estimate is possible from the data of Table V.

TABLE VI

HEAT CAPACITY PER GRAM OF BOUND WATER IN 87.5% GELATIN

Temp., °C.	25.0	0.0	−3.0	−5.0	−10.0	−20.0	−40.0	−60.0	−78.5
Heat capacity, cal.	0.0	−20.8	−23.1	−24.6	−28.3	−35.3	−46.7	−53.4	−58.4

TABLE VII

BOUND WATER PER GRAM OF DRY GELATIN IN 24% GEL

Temp., °C.	-3.0	−5.0	−10.0	−20.0	−40.0	−60.0	−78.5
Bound water							
Equation (5)	0.69	0.57	0.46	0.37	0.33	0.26	0.24
Equation (3)	0.82	0.70	0.61	0.56	—	—	—

In an 87.5% gel it is to be expected that the water is all bound. This is confirmed by the absence of any break in the curve (Curve B, Fig. 2). Subtracting the heat capacity of 0.875 gm. of dry gelatin should then give the heat capacity of 0.125 gm. of bound water. Table VI gives values per gram of bound water calculated in this way from the data taken from the smooth curves (A and B) of Fig. 2. It must be pointed out that if there are different stages or degrees of binding, the specific heat of 1 gm. of bound water in an 87.5% gel is not necessarily the same as the specific heat of 1 gm. of bound water in a 24% gel. It is probable however that any difference would be insignificant.

Values for bound water calculated by means of Equation (5) are given in the second line of Table VII. In the third line are shown for comparison values calculated by Thoenes' equation (Equation 3). The latter agree closely with those previously obtained in the same way by Mennie (11) and also with Moran's work (13). The figures in the second line are markedly lower. They do not appear to reach a minimum value at −20°C. as Moran found, but continue to decrease with the temperature as predicted by Briggs (5). It has been shown already that Thoenes' equation tends to give too high an estimate of the bound water. Nevertheless, the figures in the second line of Table VII require further confirmation, and work is in progress to this end. The values for the heat capacity of bound water in Table VI were derived from a relatively small difference between the experimental values of Curves *A* and *B* (Fig. 2) and hence may be subject to a considerable experimental error. Accordingly, further measurements are planned on very concentrated gels. Additional measurements are also being made on more dilute gels of different concentrations and at temperatures even lower than −78.5°C.

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INFLUENCE OF SOIL TEMPERATURE AND SOIL STERILIZATION ON THE REACTION OF WHEAT SEEDLINGS TO *OPHIOBOLUS GRAMINIS* SACC.[1]

By A. W. Henry[2]

Abstract

In this investigation Marquis wheat seedlings reacted similarly to a virulent Alberta strain of *Ophiobolus graminis* in sterilized and unsterilized soil at low temperatures, but differently at higher temperatures. At 13° C. for instance, the blighting was about equally severe in sterilized and unsterilized soil, but at 27° C. most of the seedlings were killed in the sterilized soil, while those in the unsterilized soil were only slightly attacked. The protective value to wheat of the constituents of unsterilized soil against soil-borne inoculum of *Ophiobolus graminis* appears, therefore, to vary with temperature, being under the conditions of these studies relatively slight at soil temperatures below 20° C.

Introduction

In most investigations on the effect of soil temperature on the development of plant diseases, conducted under controlled conditions, sterilized soil has been used. It seemed that the conclusions drawn from such studies might not always apply under field conditions, where the soil usually is not sterilized. Therefore, in these studies on the effect of temperature on the development of foot-rot diseases of wheat, it was decided to use both sterilized and unsterilized soil, so that the results might be compared. The data reported in this paper apply only to studies made with *Ophiobolus graminis*.

Review of the Literature

The influence of temperature on the growth of *Ophiobolus graminis* in artificial culture has been investigated by Davis (1), who found that growth occurred between 4° and 33° C. Three strains were studied. The optimum temperatures for the growth of one strain originating in New York, ranged between 19° and 24° C. and for two others from Oregon and Arkansas, between 23° and 24° C. The effect of temperature on the development of the "take-all" disease of wheat was studied at the University of Wisconsin by McKinney and Davis (6). They found that the optimum temperature range for the disease in the case of wheat seedlings was considerably lower than for the vegetative growth of the parasite, being between 12° and 16° C. Their studies were made in sterilized soil maintained at constant temperatures. The inoculum used was produced by growing the fungus on a mixture of cooked oat and barley kernels. Commenting on these studies, Jones, Johnson and Dickson (4) point out that the apparent temperature optima may shift somewhat, regardless of variations in moisture content, thus indicating that some other undetermined factors not controlled by corresponding temperature or moisture variations may have a decided influence on the occurrence of this disease. McKinney (5)

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has also observed that *Ophiobolus graminis* often "behaves in a very perplexing way at times in regard to both fruiting and infection."

Experimental Methods

The pathogenic fungus used in these studies was a virulent strain of *Ophiobolus graminis* (No. IV) isolated in this laboratory by Mr. F. R. Davies*, from wheat collected in 1930 at Athabasca in northern Alberta. The medium selected for the production of inoculum consisted of typical black soil of the Edmonton district plus 10% of corn meal. The fungus grew well on this medium and caused severe damage to wheat seedlings when added to the soil. The inoculum was produced in small Erlenmeyer flasks, each containing 50 gm. of the sterilized soil-cornmeal mixture plus 27 cc. of distilled water. The fungus was allowed to develop at room temperature on this medium for about three weeks, when the contents of each flask were added at seed level to a 6-in. pot of soil. Half of the pots of soil had been sterilized for four hours at 15 lb. pressure, while the other half had not been sterilized. Marquis wheat kernels previously surface sterilized with a formaldehyde solution (1:320) and washed with water, were then sown at the rate of 25 kernels per pot and covered with about an inch of soil. The pots were then embedded in moist sand in the cans of soil-temperature tanks of a type similar to those in use at the University of Wisconsin (4). In each tank there were four pots of unsterilized soil, three pots of sterilized soil which received inoculum and one pot of sterilized soil to which no inoculum was added, and which served as a check. The four temperature tanks available were maintained at average soil temperatures of 13°, 18°, 23° and 27° C. respectively, for a period of 25 days, when data were taken on the disease reaction of the wheat seedlings.

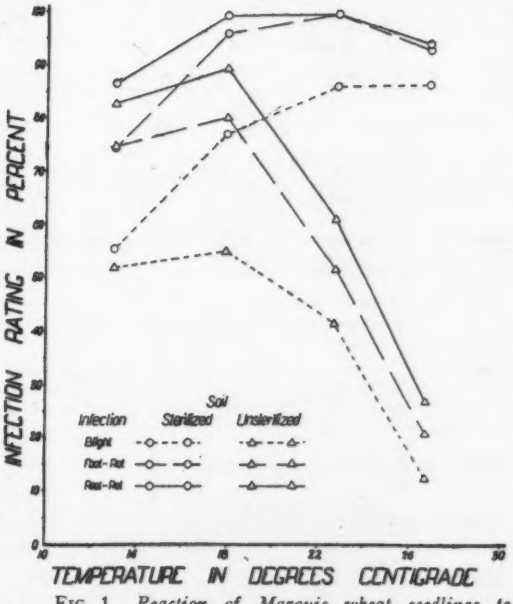


FIG. 1. *Reaction of Marquis wheat seedlings to Ophiobolus graminis at different soil temperatures in sterilized and unsterilized soil.*

Results

At the conclusion of the experiment, the following notes were taken on each seedling: height in cm., degree of blighting of the shoot, degree of rotting of the basal part of the stem

* *Graduate assistant in Plant Pathology.*

or foot, and the degree of rotting of the roots. The three latter notes were taken according to the system used by McKinney and Davis (6), the average figures being recorded as percentage. The summarized data are shown in Table I and illustrated graphically in Figs. 1 and 2. The actual effect on the seedlings of the different treatments is shown in Plate I.

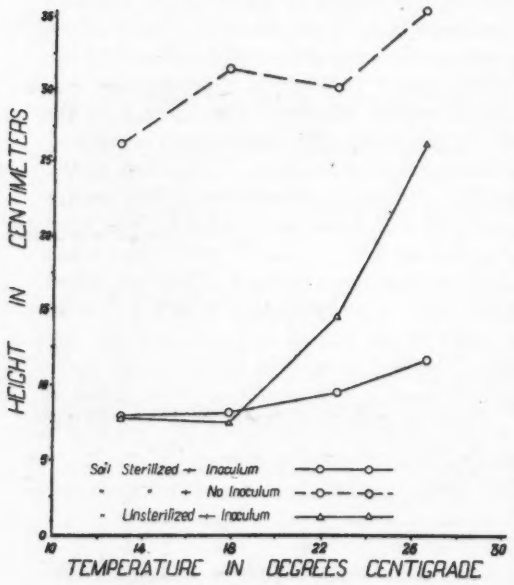


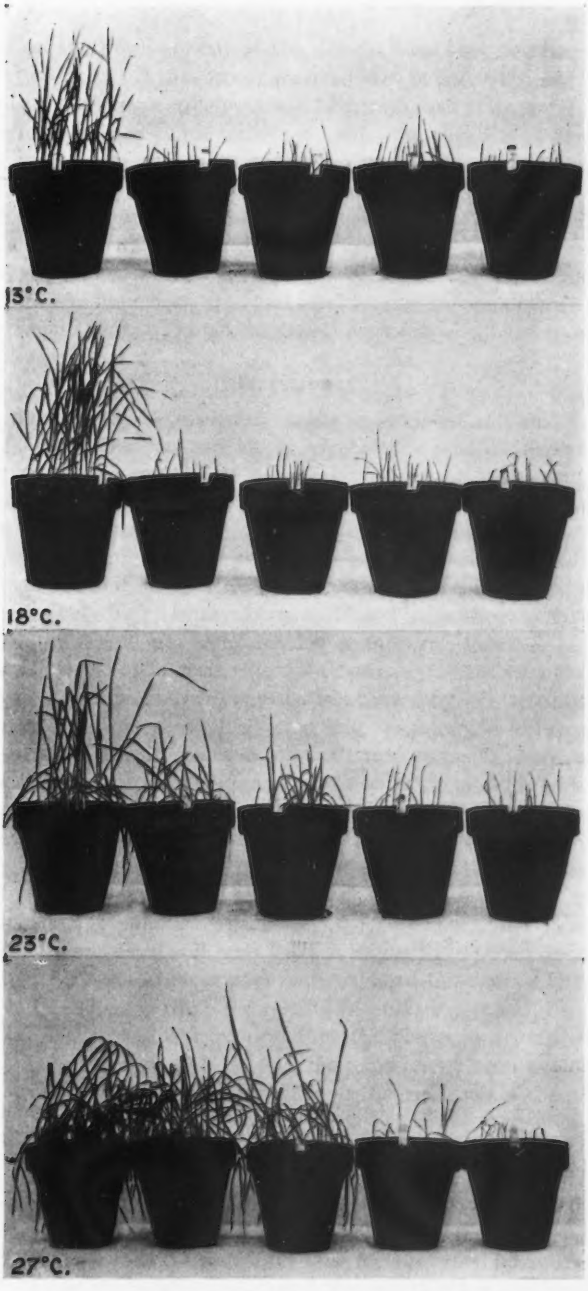
FIG. 2. *Effect of Ophiobolus graminis on the height of Marquis wheat seedlings in sterilized and unsterilized soil kept at different temperatures.*

In sterilized soil the fungus caused severe damage at all four temperatures, namely, at 13°, 18°, 23° and 27° C., but in unsterilized soil there was much less damage at the higher temperatures. At 13° C., however, there was little if any difference between the reaction in sterilized and unsterilized soil. There was a greater difference at 18° C. in the amount of blighting and lesioning, but practically none in height. At 23° C. there was a marked difference and at 27° C. a very marked difference in amount of blighting and lesioning and in the height and vigor of the plants, the healthiest and tallest plants developing in the unsterilized soil. At 27° C. most of the seedlings in the sterilized soil were dead when notes were taken at the end of 25 days, whereas those in the unsterilized soil were almost as vigorous as the uninoculated ones of the check.

TABLE I

REACTION OF MARQUIS WHEAT SEEDLINGS TO *Ophiobolus graminis* (No. IV) AT DIFFERENT SOIL TEMPERATURES, IN STERILIZED AND UNSTERILIZED SOIL

Notes		Sterilized soil				Unsterilized soil			
		13°C.	18°C.	23°C.	27°C.	13°C.	18°C.	23°C.	27°C.
Height of plants, cm.	Inoculated	7.9	8.0	9.4	11.6	7.8	7.3	14.4	26.1
	Check	26.1	31.4	30.2	35.4	—	—	—	—
Blight rating, %	Inoculated	55.3	76.5	85.6	85.7	51.8	54.9	40.3	12.2
	Check	0.0	0.0	0.0	0.0	—	—	—	—
Foot-rot rating, %	Inoculated	74.2	95.5	98.9	92.1	74.4	79.7	51.9	20.3
	Check	4.0	0.0	2.0	0.0	—	—	—	—
Root-rot rating, %	Inoculated	86.5	99.0	98.9	93.2	82.5	89.0	60.6	26.6
	Check	0.0	0.0	0.0	0.0	—	—	—	—



CHECK | UNSTERILIZED SOIL | STERILIZED SOIL

Relative amount of blighting of Marquis wheat seedlings in sterilized and unsterilized soil kept at different temperatures, following inoculation with Ophiobolus graminis.

The effect on the development of the disease may best be observed by an examination of Fig. 1. If the curves are examined in pairs representing blight, foot-rot, and root-rot respectively, it will be noted that in each case the trend of the curve for sterilized soil is quite different from that for unsterilized soil. It would appear that the effect of temperature was overshadowed by some other factor or factors in the unsterilized soil, at the higher temperatures.

It may perhaps be wondered why no checks on unsterilized soil were included in the experiment. This was simply because of lack of sufficient space. However, in experiments conducted since, with the same and similar soils, both kinds of checks have been used and no differences of importance have been observed between them. The above experiment has also been repeated with almost identical results.

Discussion

As to the discrepancy between the results in sterilized soil and those in unsterilized soil, the writer has previously suggested a possible explanation (3), namely, that meteorological factors may influence the development of the natural soil microflora, which in turn may affect the development of the pathogene and the disease caused by it. Thus it would only be necessary to assume that certain antagonistic saprophytes in the soil, several of which have been reported by Sanford and Broadfoot (8), are more active in inhibiting *Ophiobolus graminis* at relatively high temperatures and have little effect at temperatures below 20° C. It should be noted in this connection, however, that Jones, Johnson and Dickson (4) in their extensive summary of the effect of soil temperature on the development of plant diseases caused by soil-borne pathogenes, have also pointed out the possibility "that any variations in soil environment, in addition to their direct relations on host and parasite, may have some influence upon the general development of the soil flora and fauna which may in turn indirectly affect the phytopathological results." In general, however, they considered that this was not a matter of primary importance. Field results from plantings made on different dates, and therefore exposed to different temperatures, in several instances checked quite closely with those from the more exact greenhouse experiments. In the case under consideration, however, had only sterilized soil been used, it might have been concluded that severe seedling injury would occur under natural conditions at soil temperatures as high as 27° C., whereas from the results in unsterilized soil, this does not seem probable. It is possible of course that the plants in unsterilized soil at 27° C. might have succumbed later had the experiment been prolonged, but from the amount of lesioning present and from the condition of the plants, this hardly seems likely.

Although attempts to produce the disease in the field by applying artificial inoculum to the soil have frequently failed, the disease does occur in severe form under natural field conditions. Its severity, however, fluctuates widely in different districts and from season to season. Evidently the proper balance between the various factors necessary for severe infection occurs only occasionally. From the results reported, it appears that low soil temperatures

would be especially important in the production of severe infection under field conditions because of the fact that the soil is not sterilized, but if the soil were sterilized, low temperatures would not be necessary for severe infection with the strain of *Ophiobolus graminis* used in these studies.

It is possible that the results presented may help to explain the difference in severity of "take-all" of spring wheat in different geographical regions. For instance, observations made during the past ten years indicate that "take-all" is much more destructive to spring wheat in western Canada than in the United States. In fact, the disease has only recently been found in spring wheat in the United States according to Fellows and Hungerford (7) who report a severe outbreak in southern Idaho in 1930. It has been known in western Canada, on the other hand, since 1923 (2) and is now considered one of our most destructive diseases of spring wheat. These observations may possibly be explained by the fact that soil temperatures in western Canada are, at least at times, appreciably lower during the growing season than they are in the spring-wheat regions farther south in the United States. In the case of winter wheat the situation is somewhat different. Severe damage occurs in the United States as well as in western Canada, though the amount of winter wheat grown here is relatively small. However, in both countries, winter wheat is normally exposed during part of its life to low soil temperatures, and it may be at some time during this period that infection chiefly takes place.

It is realized that, besides those mentioned, there are probably other important factors concerned in determining the severity of infection caused by soil-borne inoculum of *Ophiobolus graminis* and other pathogenes. The amount of inoculum present, for instance, may be one of them. However, if we confine our attention to the factors discussed above, namely, soil temperature and the activities of the saprophytic organisms of the soil, the situation is sufficiently complex to make it unsafe to draw general conclusions for all soil-borne pathogenes. What might apply to one might not apply to another. It does not necessarily follow, for example, that the soil saprophytes which are antagonistic to *Ophiobolus graminis* affect other pathogenes in a similar manner. It is believed, however, that it has been demonstrated in at least one instance that disease reaction may differ markedly in sterilized and unsterilized soil at certain temperatures, and be approximately the same at other temperatures. In view of this, and because of the fact that field soils usually are not sterilized, it would seem desirable, in studying the influence of soil temperature on plant diseases, to at least check the results of experiments performed with sterilized soil, by repeating the experiments using the same soil without sterilizing it.

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VEGETATIVE PROPAGATION OF WILD OATS, *AVENA FATUA*, AND OTHER ECONOMICALLY IMPORTANT SPECIES OF *AVENEAE* AND *HORDEAE*[1]

By L. E. Kirk[2] and T. K. Pavlychenko[3]

Abstract

Following the discovery that small sections of wild oats seedlings could become rerooted and develop into normal plants, a study was undertaken to determine from what part or parts of the seedlings new growth can originate, and whether cultivated oats, wheat, barley and spring rye, would behave in a similar manner. Special attention was given to the problem of vegetative propagation in wild oats as a factor in the control of this weed.

Small sections of wild oats seedlings, one inch in length and containing the coleoptile node, became rerooted under favorable conditions and produced fully developed plants. Cultivated oats behaved in a similar manner to wild oats in this respect, but the latter produced the more vigorous growth.

When land is infested with wild oats and is plowed shallow, or cultivated shortly after the seedlings have emerged from the soil, a considerable proportion of them, under certain conditions, may produce new plants by vegetative regrowth. The extent to which this may occur in the field depends largely on the soil moisture as well as on other conditions which facilitate rerooting, such as shallow plowing, and packing when conditions for plant growth are favorable.

With young seedlings of wild oats at time of emergence, regrowth occurred mostly from a small area located between ground level and one inch below the surface. The same was found to be true of cultivated oats. With seedlings of wheat, barley and spring rye, at time of emergence, regrowth originated only from nodal tissue close to the seed. The difference in this respect, between oats, *Aveneae*, on the one hand, and wheat, barley and rye, *Hordeae*, on the other, is due to the fact that the area of elongation in oats is the mesocotyl, whereas in other cereals it is the first internode.

At later stages in seedling development of both oats and the other cereals, nodes which were capable of regrowth developed immediately below and above the ground level. With older seedlings in the first-, second-, and third-leaf stage, the youngest node above ground, which is the one nearest the soil, had the greatest power of regrowth.

Introduction

Weeds have become a serious problem in western Canada. They constitute a factor that has persistently forced upward the cost of production. About three years ago the feeling became general among leading agriculturists that more definite action should be taken to combat the weed menace, and this feeling became intensified when depressed prices of farm products made urgent the need for decreased costs of production.

It was decided that a general campaign of education and research work on weeds should be initiated. Existing methods of weed control were, for the most part, based upon general observations. It was believed that more effective methods of control for the different weeds could be developed if

[1] *Manuscript received May 28, 1932.*
Contribution from the Field Husbandry Department, University of Saskatchewan, with financial assistance from the National Research Council of Canada.

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precise information on the outstanding biological features of each weed species was available. As a part of the weed campaign, a research program was initiated at the University of Saskatchewan in 1930, with the co-operation and financial assistance of the National Research Council of Canada, for the purpose of making fundamental biological studies on the most important weed species. A prominent place in these investigations was given from the beginning to wild oats, since this is one of the most serious weeds in the grain growing areas.

In 1930, during the course of an experiment on the effect of weathering on the germination of wild oats seeds in soil, it was accidentally observed that seedlings could be cut in two and that some of the cut portions would produce new roots and grow into normal plants.

Soil and wild oats seeds were thoroughly mixed and placed in boxes 7 in. deep with wire screen bottoms, and buried to soil level. These boxes were to be brought in at different dates, one at a time, and germinated to determine how many of the seeds at different depths, which did not germinate at once, would grow later.

After one month of weathering in the ground, one box was brought in, the soil in it cut horizontally into seven one-inch layers by means of a special device, and the number of germinated seeds in each layer was recorded. Those seeds in each layer of soil which had not germinated were carefully sorted out, counted, mixed again with the soil of the layer in which they had been found, and placed in separate trays to be observed for a longer time in a greenhouse. At this time most of the seeds at all depths had germinated. Some seedlings thus produced had emerged above the surface while others, especially those at the lower depths, were only partly grown. Since the soil was cut into layers, all the seedlings were cut into one-inch sections, as shown in Fig. 1. Each layer of soil contained, therefore, not only a known number of ungerminated seeds, but also cut portions of the seedlings.

A germination count was made 14 days later when it was found that a few more seeds had grown, but the number of new plants exceeded the number of seeds. In the top layer of soil only 23 seeds were left, but 88 new plants had emerged. The numbers were not so large from the lower layers, but there was still a disproportionate number of seedlings. An examination of the root system of the plants showed that the majority of them had grown from the small sections of the seedlings left in the soil. Several of these vegetatively reproduced plants were transplanted into nine-inch flower pots and allowed to grow to maturity. Fig. 2 shows one of these which developed an extensive root system, attained a height of 42 in., produced four normal tillers, and matured seeds.

The discovery that cut sections of wild oats seedlings could establish themselves as new plants at once suggested that the same phenomenon might be of common occurrence in the field, and that it might be of practical importance in connection with the control of this weed. It also indicated a problem of considerable theoretical interest.

Literature Review

Vegetative propagation in perennial grasses has been extensively studied by forage crop workers, horticulturists and greenkeepers all over the world. References to this subject in the literature are very numerous. Perennial grasses, possessing various types of underground stems and bearing numerous overwintering buds, are easily propagated by small sections of their subterranean parts. The vegetation which results from such cuttings is as vigorous as that produced from seed.

An entirely different situation exists with regard to the annual grasses. The only cases where the writers were able to find even indirect indication as to the possibility of vegetative regrowth among these are given below.

Percival (1) refers to Miller's experiment in which tillers were separated from the mother wheat plant, together with their adventitious root system; these, when transplanted, grew to maturity. Shirreff (1) repeated Miller's experiment with still better results. In 1908 and 1909 Demchinsky considerably increased the yield of a single wheat plant by covering the stems above ground with soil in order to stimulate tillering. Tillers of several orders were produced in succession. In 1928 a very interesting experiment was conducted by Simmonds (2, 3). He studied the effect of severing the seminal roots and loss of the whole underground part below the crown node, on the further development of the injured plant. He concluded that "seminal root amputations and subcrown internode cuts cause weak tillering and delayed ripening." The plants grew, but were somewhat retarded in their development by the injury.

It will be well to keep in mind, however, that in all these cases the parts which continued to grow had all the principal organs of a normal plant, that is, roots, leaves and stems, established and well developed before they were detached from the mother plant. In Demchinsky's case, the tillers were not even detached from the original seed plant. In no case could it be considered that the authors were dealing with material produced by means of vegetative cuttings.

Scope of the Work

Wild oats, cultivated oats, wheat, spring rye and barley were chosen for experimentation. These include the main economically important annual species of *Aveneae* and *Hordeae* in this country. Experiments were carried on both in the greenhouse and under field conditions for the purpose of securing answers to the following questions:

1. Which particular parts of the seedlings of wild oats are capable of vegetative propagation?
2. What particular stages of plant development are most favorable for regrowth?
3. Are wild oats plants peculiar in this respect or do cereal crop plants behave in a similar manner?
4. To what extent are wild oats propagated vegetatively in the field, and how is regrowth related to the control of this weed?

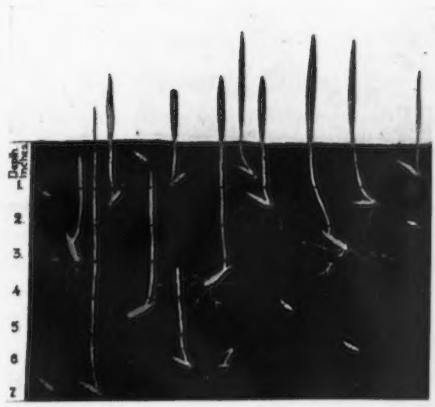


FIG. 1. *Method of cutting the soil which resulted in severing the wild oats seedlings into one-inch sections.* FIG. 2. *Specimen of wild oats grown from a one-inch section of a young seedling.* FIG. 3. *Wild oats plant propagated vegetatively from the uppermost one-inch section cut from a seedling before emergence, excavated 18 days after planting.* FIG. 4. *Wild oats plants produced vegetatively from one-inch sections of seedlings (from left to right) cut on the first, fourth, sixth, tenth and thirteenth day after emergence. In each case the section included the top and 1 in. of the underground stem.* FIG. 5. *Wild oats plant produced vegetatively from a one-inch section of a seedling four days after emergence, taken at a depth from 2 to 3 in. below the surface.* FIG. 6. *Wild oats plant produced vegetatively from the stem cut below the first node above the soil level. The cutting was planted on the thirteenth day after emergence and excavated on the twenty-fourth day.*

A. Vegetative Propagation in Wild Oats, *Avena Fatua*

1. Regrowth from One-inch Sections of Wild Oats Seedlings Cut Before Emergence

Wild oats seeds were planted in greenhouse flats 7 in. in depth. Just before emergence, 50 seedlings were carefully separated from the soil by removing one side of the box and breaking away the earth. The seedlings were then cut into one-inch sections, beginning from the top, and all those of the same depth were transplanted into separate flats and covered with ½ in. of soil. In every case the top portion was designated "Section 1." Those taken at depths of 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, and 6 to 7 in. were designated Sections 2, 3, 4, 5, 6 and 7 respectively. Care was taken not to use plant material containing adventitious roots developed before the seedlings were cut into sections in order to avoid confusion, on the one hand, between genuine vegetative regrowth, where all parts of the re-established plant came from tissues of the cut seedlings, and on the other, normal tillering, where shoots developed before they became detached from the seed-piece. Fig. 3 shows a normal plant developed from Section 1. The data secured from this experiment are shown in Table I.

TABLE I

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS OF WILD OATS SEEDLINGS CUT BEFORE THEY EMERGED ABOVE GROUND. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.*
No. of cuttings survived	11	2	0	0	0	0	0
Height, in.	8	6	0	0	0	0	0
Per cent survived	22	4	0	0	0	0	0

NOTE:—*Depth of seeding, 7 in. Records taken 40 days after the plants were sectioned.*
**This section included the seed and seminal roots.*

The results from this experiment indicate that:

1. A large number of one-inch sections of wild oats seedlings cut before emergence developed new root systems and stems, and grew as normal plants.

2. Regrowth was confined to portions of seedlings situated from 0 to 2 in. in depth.

3. That portion of the seedling situated from 0 to 1 in. in depth was the one which produced the majority of new plants.

4. Sections taken at a depth of 1 to 2 in. produced new plants in only a few cases. As a rule these were less vigorous than those developed from sections taken at a shallower depth.

2. Regrowth from One-inch Sections of Wild Oats Seedlings Grown at Different Depths and Cut One Day After Emergence

In this experiment seeds were planted at depths of 5, 6 and 7 in. The seedlings were cut one day after emergence, and they were approximately three days older than those used in the preceding experiment. Section,

TABLE II

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS OF WILD OATS SEEDLINGS GROWN AT 5, 6 AND 7 IN. IN DEPTH, AND CUT ONE DAY AFTER EMERGENCE. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.
Seeded 5 in. deep							
No. of cuttings survived	45	2	2	0	0		
Height, in.	13	13	8	—	—		
Per cent survived	90	4	4	0	0*		
Seeded 6 in. deep							
No. of cuttings survived	19	3	1	1	0	0	
Height, in.	12	10	6	6	—	—	
Per cent survived	38	6	2	2	0	0*	
Seeded 7 in. deep							
No. of cuttings survived	17	0	1	0	0	0	0
Height, in.	11	—	—	—	—	—	—
Per cent survived	34	0	2	0	0	0	0*

**The lowest section in each case included the seed and seminal roots.*

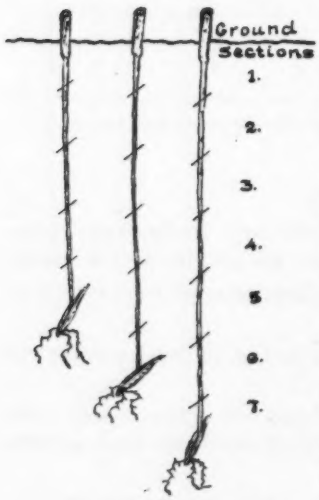


DIAGRAM 1. *Method of cutting sections in Experiments Nos. 1, 2 and 3.*

therefore, included 1 in. of the underground stem and also the above-ground portion (see Diagram 1). All other sections were 1 in. in length. The results obtained in this experiment are shown in Table II.

The results obtained from this test seemed to indicate that a difference in depth of seeding did not appreciably affect the location of that portion of the underground stem which was capable of regrowth after being cut into one-inch sections. At all three depths of seeding, only Section No. 1 grew readily. In a few cases, Sections Nos. 2 and 3 re-established themselves as new plants, but the number of these was very small. Considering the fact that, in the first experiment, none of the sections below 2 in. grew, while in this test, where the plants were about three days older, they did so, it might be thought that the age of seedlings had some effect upon the activity of the meristematic tissue of sections located at the lower depths. Otherwise, the results from this experiment are in good agreement with those obtained in the previous test.

3. *Effect of Age of Seedlings on the Number of New Plants Produced from Cut Sections*

This experiment was made to determine how the particular stage of plant development would affect the amount of regrowth. Seeds were planted 7 in. deep, but the cuttings were made 1, 4, 6, 10 and 13 days after emergence. The material was handled as described in the preceding experiments.

The results are shown in Table III, and a few typical plants are shown in Figs. 4 and 5. The method of cutting sections was similar to that shown in Diagram 1.

TABLE III

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS CUT FROM SEEDLINGS OF DIFFERENT AGES. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION AT EACH AGE

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.
One day after emergence							
No. of cuttings survived	46	1	0	0	0	0	0
Height	13	6	—	—	—	—	—
Per cent survived	92	2	0	0	0	0	0
Four days after emergence							
No. of cuttings survived	10	0	0	0	0	0	0
Height	8	—	—	—	—	—	—
Per cent survived	20	0	0	0	0	0	0
Six days after emergence							
No. of cuttings survived	9	0	0	0	0	0	0
Height	11	—	—	—	—	—	—
Per cent survived	18	0	0	0	0	0	0
Ten days after emergence							
No. of cuttings survived	13	0	0	0	0	0	0
Height	14	—	—	—	—	—	—
Per cent survived	26	0	0	0	0	0	0
Thirteen days after emergence							
No. of cuttings survived	27	2	0	0	0	0	0
Height	15	7	—	—	—	—	—
Per cent survived	54	4	0	0	0	0	0

NOTE:—*Records taken 40 days after plants were sectioned.*

Fig. 4 shows plants which were grown from the top sections, including 1 in. of the underground stem, cut from seedlings 1, 4, 6, 10 and 13 days after emergence. These were carefully selected so as to represent fairly the plants grown in each series. Starting from left to right one may see that the first specimen (1 day after emergence) showed vigorous top and root development. The second plant (4 days after emergence) was somewhat inferior in these

respects. Specimens 3 and 4 (6 and 10 days after emergence) were very weak as compared with the two preceding ones. Specimen No. 5 (13 days after emergence) exhibited again a well-developed root system and top growth.

The difference in behavior of cut portions taken from material of different ages was so striking that it was found necessary to repeat the experiment. In both cases, however, the results were practically the same. Microscopical examination of the seedlings of different ages showed that the nodal tissue of a plant one day after emergence had hardly become differentiated sufficiently to be distinguished from ordinary stem tissue. At the same time, the outermost stem tissue surrounding the nodes at this stage was very tender. Four days after emergence, the nodes were quite apparent, but while the outermost layers of cells indicated further progress in differentiation, they were still tender and soft. In six- and ten-day material, the secondary roots could be observed starting at the nodal region inside the stem. The outermost tissue of the stem at this stage had hardened sufficiently to exert considerable resistance to the secondary roots pushing through it. The secondary roots of seedlings 13 days after emergence, in nearly all cases, had already emerged from the stem tissue in the form of tiny protuberances around the nodes.

When very young seedlings were cut into small sections, growth appeared to be active in the meristematic tissue of the nodes. The meristem of the young seedlings, being in a very active stage of cell division, differentiated rapidly into stem and roots. These latter easily pushed through the tender tissue of the outermost layers of the stem, reached the soil, and began to support the new growth. Nodes of plants which were sectioned four days after emergence, had, for the most part, passed beyond this stage of cell division, and moreover, the external layers of the original stem tissue seemed to offer much greater resistance to the new roots pushing through them than they did at an earlier stage. These are, perhaps, the two main reasons why the majority of cuttings taken from such material were dead before new roots had developed.

The secondary roots of seedlings older than 10 days after emergence were well advanced in their development at the time of cutting, and usually continued to grow after being detached from the mother plant. For this reason, sections cut from seedlings older than 10 days after emergence re-established themselves nearly as readily as those taken from seedlings less than 4 days old. Sections taken at greater depths as a rule died, but occasionally one of them survived and produced a healthy plant (see Fig. 5).

The results of the experiment to determine the effect of age of seedlings on vegetative regrowth in wild oats may be summarized as follows:

1. The largest number of plants and the strongest were obtained from sections of young seedlings up until 4 days after emergence.

2. Seedlings which were sectioned 4 to 10 days after emergence gave fewer and weaker plants than younger seedlings.

3. Sections cut 10 to 13 days after emergence produced a higher percentage of plants than those from 4 to 10 days.

4. In all cases, sections of the stem nearest the soil surface produced the greatest number of new plants.

4. Regrowth from the Stem Above Ground

In preceding experiments it was evident that vegetative regrowth occurred readily from one-inch sections cut from seedlings of different ages. They showed also that regrowth is most likely to occur from the part which is located in the first inch below the surface. There was not sufficient information, however, to indicate whether new plants could be produced from sections of seedlings which did not contain nodal tissue, that is, from internodes, though of course there was every reason to believe that this would not be possible. The point could not be decided from the preceding experiments for the reason that nodal tissue had not been sufficiently differentiated so that it could be observed at the time when plants were sectioned. Definite information was obtained on this point in this and other experiments, which showed that nodes were the only regions from which both new roots and stems could originate.

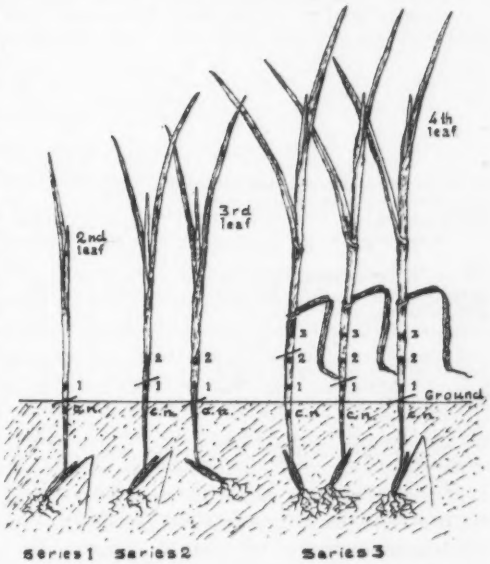


DIAGRAM 2. *Method of sectioning wild oats seedlings at different stages of growth in relation to the nodes above ground (c.n. = crown node).*

Because it was not possible to locate the nodes in very young seedlings, it was decided to use older plants from which sections of the stem above ground could be taken, as it was found that the nodes could be identified at any time after the second leaf made its appearance. With such material it was possible to plant cuttings in such a way that only internodal tissue was buried under the ground. In other cases, they were planted with at least one node covered with soil.

Three series of plants were grown in this experiment, each sectioned at different stages of development as shown in Diagram 2, the experiment being done in duplicate. In one case the top portions were transplanted so that only the internode was covered with soil, while in the other case, corresponding top portions were planted so that the bottom node in each case was placed under the surface.

Data on the number of sections which became re-established as new plants were recorded at intervals of 20 days after they were sectioned.

The data from the 300 sections which were planted so that only the internode was buried are not tabulated as none of them grew, but those sections

which were planted with their lowest nodes covered with soil, in many cases re-established themselves as new plants (see Table IV). This is substantial evidence that regrowth occurs only from nodes and not from internodes. Fig. 6 shows a plant produced from one of these cuttings.

TABLE IV

NUMBER, HEIGHT, AND PER CENT OF NEW PLANTS PRODUCED VEGETATIVELY FROM THE TOP PORTIONS OF WILD OATS SEEDLINGS CUT OFF BELOW THE 1ST, 2ND, AND 3RD NODES ABOVE GROUND, AND PLANTED WITH THE LOWEST NODE BURIED UNDER THE SURFACE

Series	Position of node above ground	No. of tops planted	No. of plants survived	Height, in.	Per cent of plants survived
1					
1st node above ground. Section cut on the day when the second leaf appeared	1st	50	38	13	76
2					
1st and 2nd nodes above ground. Section cut on the day when the 3rd leaf appeared	2nd	50	3	11	6
	1st	50	12	16	24
3					
1st, 2nd and 3rd nodes above ground. Section cut when the 4th leaf appeared	3rd	50	0	—	0
	2nd	50	2	14	4
	1st	50	11	18	22

NOTE:—*Records taken 40 days after plants were sectioned.*

A closer examination of the data presented in Table IV shows that in each series it was the first node above ground and the last to emerge that produced the largest number of new plants. Older nodes higher up on the stem gave very few plants. It was also apparent that the material taken from the youngest plant, having only one node above ground, rerooted much more readily, and produced three times as many plants as that secured from older seedlings, which had two or three nodes above ground.

Field Experiments

From experimental work on the problem of regrowth of wild oats under greenhouse conditions it is obvious that this weed may be propagated vegetatively. Thus, under certain conditions, there is the possibility that plants may re-establish themselves after having been plowed down or cultivated. The phenomenon, therefore, may be one of considerable economic importance. For the purpose of studying this particular problem, certain tests were undertaken under field conditions throughout the season of 1931. Owing to the fact that the season was extremely dry, it was found necessary to water the soil before the cuttings were transplanted. When the heat was very great, it was found useful to protect the newly planted cuttings by shading them with wrapping paper for one or two days. Even these precautions did not furnish conditions nearly as favorable as those which occur in the spring, when regrowth is most likely to occur under field conditions.

TABLE V

NUMBER OF NEW PLANTS PRODUCED FROM THE TOPS OF WILD OATS SEEDLINGS CUT OFF AT DIFFERENT STAGES OF PLANT DEVELOPMENT AND PLANTED IN THE FIELD, 10 SEEDLINGS BEING USED IN EACH CASE

Type of sectioning	Days from emergence											
	1	1	1	2	3	4	5	12	13	21	25	32
	Number of re-established plants											
Tops cut at soil surface	0	0	0	0	1	1	0	0	1	2	1	0
Tops cut 1 in. below surface	10	10	10	10	9	9	4	10	8	9	10	10

5. Vegetative Propagation of Wild Oats in the Field, from Sections Cut at Various Stages of Plant Development

Wild oats seeds were planted in the field in eight-foot rows at a depth of six inches. The seeding was repeated throughout the season at 15-day intervals in order to provide plant material of different ages for experimentation. At the time of emergence, and at various later dates, some seedlings were cut off at ground level and others at one inch below the surface. All the top portions were planted, while the seed parts, in this case, were discarded. The experiment was repeated many times during the summer with similar results. In Table V a typical sample of the results is given to illustrate the extent of regrowth when the plants were sectioned on the 1st, 2nd, 3rd, 4th, 5th, 12th, 13th, 21st, 25th and 32nd day after emergence. The data from this experiment show that, in the field as well as in the greenhouse, seedlings as old as 32 days from emergence can reproduce vegetatively from the top sections cut off a little below the soil surface.

6. Regrowth from One-inch Sections of the Underground Stems of Wild Oats Seedlings Cut at Various Stages of Plant Development

In experiment 5 we were dealing with the top portions of the seedlings cut at different stages of plant development. In this experiment plant material from the same plots was used, but each plant was severed at the soil level and the underground portions were cut into sections one inch in length, beginning at the top. This made six sections for each plant. When these were replanted it was found that practically none of them would grow except the uppermost, that is, the sections in the first inch below the surface. The results indicate that regrowth from short underground sections of wild oats stems can occur under field conditions, and that the nodal tissue is found mainly at this particular depth. The results are shown in Table VI.

7. Regrowth from the Stem above Ground, when the Latter is not Detached from the Original Root System and is Covered with Soil at Various Stages of Plant Development

In experiment 4 the behavior of the top portions of seedlings sectioned at different stages of development and grown under greenhouse conditions was observed. In this present experiment a similar test was made in the field, but

TABLE VI

NUMBER OF NEW PLANTS PRODUCED FROM THE UNDERGROUND SECTIONS OF WILD OATS SEEDLINGS CUT AT DIFFERENT STAGES OF PLANT DEVELOPMENT, 10 PLANTS BEING USED IN EACH CASE

Depth, in.	Days from emergence when sectioned											
	1	1	1	2	3	4	5	12	13	21	25	32
	Number of new plants produced											
0–1	9	10	8	7	3	8	2	7	6	6	7	9
1–2	0	1	0	0	0	0	0	0	0	1	0	0
2–3	0	0	0	0	0	0	0	0	0	0	0	0
3–4	0	0	0	0	0	0	0	0	0	0	0	0
4–5	0	0	0	0	0	0	0	0	0	0	0	0
5–6	0	0	0	0	0	0	0	0	0	0	0	0

in a slightly different way. Several rows of wild oats were covered with soil at various stages of plant development until only the leaf tips were exposed to light. In the fall, a random sample of 20 plants was taken from each row. Each plant was examined with respect to the number of new root systems and corresponding new plants produced. In this experiment also it was evident that regrowth occurred only at the nodes. The results are presented in Table VII and specimens of rooted stems are shown in Fig. 7.

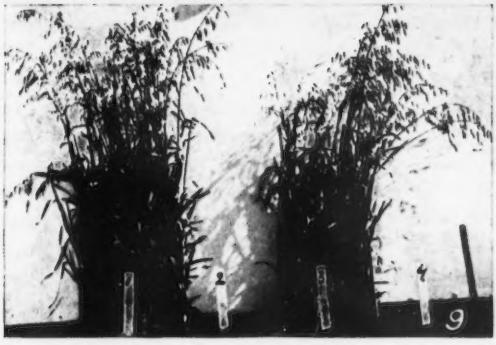
TABLE VII

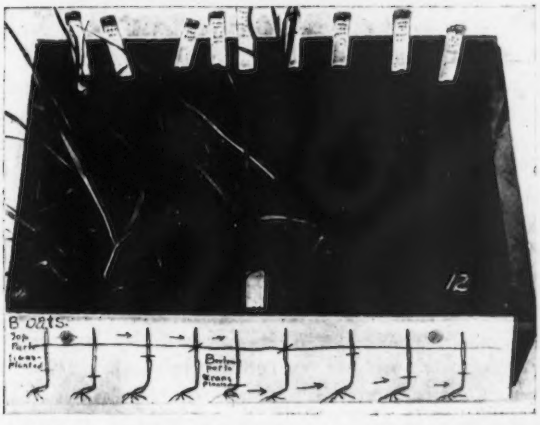
REROOTING FROM ABOVE-GROUND NODES OF WILD OATS WHEN THE TOPS WERE COVERED WITH SOIL AT DIFFERENT STAGES OF PLANT DEVELOPMENT. TWENTY PLANTS EXAMINED IN EACH CASE

Stage of growth	No. of plants producing new root systems and stems at				Per cent of rerooted plants
	1st node above ground	2nd node above ground	3rd node above ground	4th node above ground	
1. 2nd leaf	1	2	3	9	75
2. 3rd leaf	1	1	3	11	55
3. 4th leaf	2	2	5	17	85

8. Tillering from Nodes of Wild Oats Located Deeply Underground, when the Top Growth was Inhibited

In experiments 4, 5 and 7, it was possible to demonstrate clearly that if sections of the above-ground stem are subjected to favorable conditions for regrowth, the latter invariably takes place from nodes. In experiments 1, 2, 3 and 6, it was found that regrowth can occur from sections of the underground stem as well, yet it was not possible to demonstrate satisfactorily in the latter case whether or not it originated only from nodal tissue. On this account, an attempt was made to force the underground stem of wild oats into active growth by inhibiting the top growth, in which case it might be possible to observe where new roots or shoots occurred.





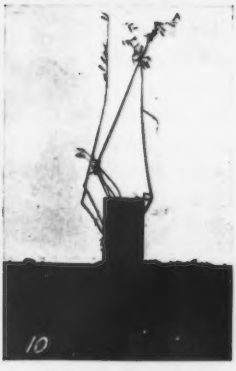


FIG. 7. *Rerooting and tillering at the nodes above ground from stems of plants that had been covered with soil. From left to right: wild oats, cultivated oats, barley, wheat.* FIG. 8. *Underground portion of wild oats plant, grown from a depth of 10½ in. showing buds and secondary roots, developed from nodes at 1, 1½ and 5 in. in depth. Fig. 9, Wild oats plants vegetatively produced from the top and underground sections of the stems. From left to right: 1, tops cut 1 in. under the surface (survival 100%); 2, tops cut at ground level (survival nil); 3, one-inch sections taken at 0 to 1 in. in depth (survival 90%); 4, one-inch sections taken at 1 to 2 in. in depth (survival nil).* FIG. 10. *A wild oats plant supported by a single primary root from the two-leaf stage until maturity.* FIG. 11. *A wild oats plant supported by a single secondary root from the seedling stage until maturity.* FIG. 12. *Top portions (left) and seed portions (right) of Banner oats showing the plants which became rerooted and survived. Cultivated oats and wild oats behaved in a similar manner.*

When fully developed, but before maturity, the top growth was cut at soil level. The new shoots which came up soon after were cut again and again. On the 20th day after the first cutting a number of these plants were excavated, carefully washed, and individually examined. Fig. 8 shows a typical specimen which had produced a new root system and tillers from a node 1 in. below the surface. These were purposely cut off before taking the picture, in order to show more clearly the portion of the underground stem at lower levels. At points located at depths 1½ and 5 in., two additional root systems were developed. Examination of untreated plants indicated that these buds would have remained dormant had they not been forced into growth. Not only were new root systems developed, but shoots also appeared at points where the roots emerged. This is additional evidence that rerooting occurs only from nodes.

Fig. 9 shows the luxuriant growth produced from cut sections of wild oats seedlings, and serves to emphasize how important the phenomenon of vegetative propagation may be under certain conditions, in the practical control of this weed in the field. Row No. 1 at the left originated from the top section of seedlings cut 1 in. below the surface. Row No. 2, which produced no plants, was planted from top sections cut at ground level. Row No. 3 grew from one-inch sections taken between the soil surface and 1 in. in depth. Row No. 4, planted with sections between 1 and 2 in. in depth, did not produce a single plant in this case.

9. Regrowth of Wild Oats Seedlings in a Cultivated Field

Information obtained from the eight preceding experiments explains satisfactorily the nature of vegetative propagation of wild oats. From a practical point of view, however, the farmer is interested primarily in whether wild oats seedlings that have been plowed down are likely to grow again. An attempt was made, therefore, to do some work on ordinary farm land infested with wild oats. The conditions in the spring of 1931 proved most unfavorable, but later in the season, field conditions were simulated in the Weed Nursery by seeding wild oats thickly on a piece of land and plowing down the seedlings. This was done at the second-leaf stage (15 days after emergence, on July 12) when temperatures were high and the soil dry.

The soil was plowed 4 in. deep and the furrow left rough until fall. Most of the plants were covered but some of the leaf tips were left exposed, and in other cases the roots were exposed. Because of the dry condition a piece of the field, 100 sq. ft. in area, was watered. Five days later a light rain fell, which penetrated the soil for ¾ in., and afterwards the weather was variable with some light precipitation. The plowed block was inspected from time to time until August 20, when final records were taken, both on the watered and unwatered areas. The proportion of plants that survived was 25% in the watered plot and 0.5% in the area which had not been watered. Most of the plants having their leaf tips exposed on the watered plot survived until maturity, and produced seed. On the unwatered area most of the plants were killed. Very interesting, however, were certain plants that were entirely uprooted except

for a single primary or secondary root. These plants were left on the field in this condition until fall without any additional treatment and in all cases they survived, produced several tillers, and matured seed. Two of these are shown in Figs. 10 and 11.

This last experiment gave definite evidence that, even under unfavorable conditions of moisture and temperature, plants which have been injured by farm implements and covered with soil, or which have had nearly all their roots destroyed, can re-establish themselves and grow again. Since only 0.5% of the original number of plants on the unwatered area recovered after being plowed down, while on the watered plot 25% of these survived, it would seem that the number of plants which is likely to become rerooted under field conditions will be directly related to soil conditions which favor or retard vegetative regrowth of the cut sections. It is logical, therefore, to presume that regrowth in the field would occur most frequently following early spring cultivation, when growing conditions are most favorable, and when the work is done in such a manner as to leave the wild oats seedlings partly covered, or otherwise favorably situated for becoming rerooted.

B. Vegetative Propagation in Cereal Crops *Aveneae* and *Hordeae*

Having investigated the phenomenon of regrowth in wild oats, the authors were interested in learning whether the cereal crop plants would behave in the same manner. Some experiments were planned, therefore, with wheat, cultivated oats, barley and spring rye, in order to determine the extent to which these could be propagated from vegetative cuttings.

10. Vegetative Propagation from the Upper and Lower Portions of Wild Oats and Cereal Crop Plants

Seeds of wild oats, cultivated oats, wheat, spring rye and barley were sown in greenhouse flats at a depth of 1½ in., five flats being seeded to each crop. In one set of five flats the plants of each crop were cut in two just above the seed (series No. 1). In series Nos. 2, 3 and 4 the plants were cut ¼, ½ and 1 in. above the seed respectively. Those in series No. 5 were cut off at ground level. All plants were sectioned on the fifth day after emergence, and both the top and the lower portion of each seedling were replanted. The results from this experiment are given in Table VIII. Re-established plants are shown in Figs. 12, 13, 14 and 15.

From the results shown in Table VIII, and as illustrated in Figs. 12, 13, 14 and 15, it is evident that there was a striking difference between wild and cultivated oats on one hand, and wheat, rye and barley on the other. In both species of oats it was the top portion which grew, whereas with the other cereals it was the seed portion which produced plants. This indicates a fundamental difference in the morphology of the two tribes *Aveneae* and *Hordeae*. In several special experiments this question was fully investigated and explained as follows: The region of primary elongation of the underground stem in oats is the mesocotyl, which is located between the base of the coleoptile and the seminal roots. In wheat, rye and barley, on the other hand,

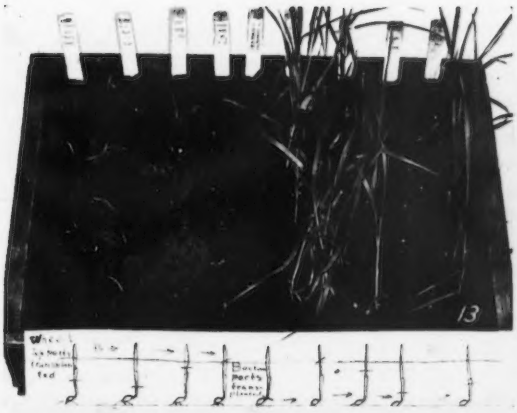


FIG. 13. *Top portions (left) and seed portions (right) of Marquis wheat showing the plants which became rerooted and survived. Wheat, rye, and barley behaved in a similar manner.* FIG. 14. *Plants produced from the top portions of cultivated oats, cut at different levels above the seed (from left to right): 1, ¼ in. above the seed; 2, ½ in. above the seed; 3, 1 in. above the seed; 4, at soil surface, the top portion in this case died, but the corresponding lower portion grew.* FIG. 15. *Plants produced from the seed portions of wheat cut at different levels below the surface. (from left to right): 1, cut at ground level; 2, cut 1 in. above the seed; 3, cut ½ in. above the seed; 4, cut ¼ in. above the seed.* FIG. 16. *Wild oats seedling two days after emergence.* FIG. 17. *Wheat seedling two days after emergence. Explanatory notes for Figs. 16 and 17: A, seminal roots; B, cotyledonary node; C, coleoptile node; D, coleoptile; E, second node on stem above the seed; G, second green leaf above coleoptile; H, first green leaf above coleoptile; I, mesocotyl; K, first true internode.*

TABLE VIII

NUMBER OF NEW PLANTS PRODUCED VEGETATIVELY FROM THE TOP AND SEED PORTIONS OF WILD OATS, BANNER OATS, MARQUIS WHEAT, PROLIFIC SPRING RYE, AND HANNCHEN BARLEY. TEN PLANTS USED IN EACH CASE

Plant	Sectioned	No. of cuttings survived		Per cent of cuttings survived	
		Top part	Seed part	Top part	Seed part
SERIES I					
Wild oats	Close to seed	10	0	100	0
Banner oats		10	0	100	0
Marquis wheat		0	0	0	0
Prolific rye		0	1	0	10
Hannchen barley		0	0	0	0
SERIES II					
Wild oats	One-quarter inch above seed	10	0	100	0
Banner oats		5	0	50	0
Marquis wheat		0	4	0	40
Prolific rye		0	4	0	40
Hannchen barley		0	2	0	20
SERIES III					
Wild oats	One-half inch above seed	9	0	90	0
Banner oats		7	0	70	0
Marquis wheat		0	8	0	80
Prolific rye		0	3	0	30
Hannchen barley		0	6	0	60
SERIES IV					
Wild oats	One inch	7	0	70	0
Banner oats		6	0	60	0
Marquis wheat		0	7	0	70
Prolific rye		0	6	0	60
Hannchen barley		0	8	0	80
SERIES V					
Wild oats	At soil level	1	6	10	60
Banner oats		0	6	0	60
Marquis wheat		0	10	0	100
Prolific rye		0	6	0	60
Hannchen barley		0	10	0	100

elongation takes place in the first true internode, which is the region immediately above the coleoptile node. Figs. 16 and 17 show the fundamental difference between wild oats and wheat seedlings grown from a depth of 3 in. and excavated on the second day after emergence (see also Simmonds (2, 3)).

In the early stages of seedling development, it is the coleoptile node which produces regrowth most readily in both oats and the other cereals. In oats, however, this node is pushed up to the surface by elongation of the mesocotyl, and this is the reason why the seed portion of oats seedlings never produces new plants (see Figs. 12 and 16). In wheat, rye and barley, the coleoptile node remains close to the seed and the region of elongation above it does not become differentiated into distinct nodal tissue until the seedling is somewhat advanced in development. This explains why regrowth does not occur from the tops in these plants when they are cut a little distance above the seed (see Figs. 13 and 17). The seed portions, however, bearing the coleoptile node, are

capable of regrowth (see Fig. 13 and Table VIII). In Fig. 14 four specimens of cultivated oats are shown, three of which were developed from the top portions. In the fourth case the seedling was cut off at ground level and died, but the seed portion survived and produced a new plant. Fig. 15 shows specimens of wheat which developed from the seed portions. The top portions invariably died. This striking contrast in the behavior of the two tribes is directly associated with a difference in their morphology as explained above.

11. Vegetative Propagation from the Top and Seed Portions of Cereal Plants Sectioned at Various Stages of Development

In this experiment an attempt was made to section the plants at later stages of growth. Seedlings of cultivated oats, wheat, rye and barley were cut below the first, second and third nodes above the surface, at ground level, and at 1 in. below the surface. The plants were severed at the one-, two- and three-leaf stages. In all cases the top and seed portions were replanted.

As in the preceding test, the majority of the seed portions of very young wheat, rye and barley plants grew again, while the corresponding top portions died. Ten days after emergence the new growth from the seed portions died also. In the case of older plants, on the other hand, the top portions in many instances produced normal plants. This was especially true of the top portions which included 1 in. of stem below the surface, or the first node above ground.

From the information obtained in this experiment it is obvious that both oats and the other cereals can reproduce vegetatively, but only from the top portions, and in the later stages of development. In the case of wheat, rye and barley, this is due undoubtedly to the fact that at this stage the coleoptile nodes have passed beyond the stage of greatest cell activity. On the other hand, nodes located immediately below and above the soil surface are in a better growing condition, and hence are capable of regrowth.

With regard to the occurrence and vigor of vegetative regrowth in oats and other cereals, it is essential to point out that the former are more capable of regrowth, and the plants thus produced are always more vigorous than those of wheat, rye, or barley.

Field Experiments

12. Regrowth of Cereal Crops Under Field Conditions

Wheat, oats, barley and rye were tested for vegetative regrowth under field conditions in 1931. These were sown at a depth of 3 in. in 8-ft. rows, at intervals throughout the season, on the same dates as were used for wild oats in Experiments Nos. 5 and 6. At emergence, and at various other times, these plants were cut off both at the surface and at 1 in. below the ground. Both the top and seed portions were replanted with the results shown in Table IX.

The plant material in this experiment was sectioned on the 1st, 3rd, 9th, 12th, 24th and 32nd days after emergence. As in all previous experiments, plants of the two different tribes behaved entirely unlike in the early seedling stage, but in a similar manner from approximately 10 days after emergence.

This is exceptionally well shown in Table IX. In the case of seedlings younger than 10 days after emergence, neither oats nor the other cereals produced new plants from the tops when they were cut at the surface. Apparently no nodal tissue capable of regrowth was included in these portions. Oats plants which

TABLE IX

REGROWTH OF CEREAL PLANTS UNDER FIELD CONDITIONS, 1931. TWENTY SEEDLINGS USED IN EACH CASE*

Plant	Age of seedlings from emergence, days	Number of new plants			
		Cut at one inch below surface		Cut at surface	
		Top portion	Seed portion	Top portion	Seed portion
Wheat	1	0	4	0	6
Wheat	1	0	10	0	10
Rye	1	0	0	0	4
Rye	1	0	2	0	2
Barley	1	0	0	0	4
Barley	1	0	4	0	4
Cultivated oats	1	10	0	0	5
Cultivated oats	1	10	0	0	8
Wheat	3	0	4	0	8
Rye	3	2	0	0	0
Barley	3	0	0	0	7
Cultivated oats	3	10	0	0	4
Wheat	9	0	0	0	0
Rye	9	2	0	0	0
Barley	9	0	0	1	1
Cultivated oats	9	—	—	—	—†
Wheat	12	10	0	0	0
Rye	12	9	0	2	0
Barley	12	10	0	0	0
Cultivated oats	12	10	0	0	4
Wheat	24	0	0	0	0
Rye	24	8	0	0	0
Barley	24	—	—	—	—†
Cultivated oats	24	10	0	0	0
Wheat	32	10	0	0	0
Rye	32	9	0	0	0
Barley	32	9	0	0	0
Cultivated oats	32	—	—	—	—†

**The results from 10 plants are given in columns 1 and 2, and from the other 10 plants in columns 3 and 4.*

†Plants were damaged.

were cut at 1 in. below the surface grew again in all cases, but none of the other cereals did so. This was due to the fact that the nodal tissue in oats seedlings was pushed up to the surface by the elongation of the mesocotyl, and thus was included in the tops. In the other cereals the first (coleoptile) node remained close to the seed, while other nodes which might develop later near the surface had not, at this time, been differentiated sufficiently to initiate

new growth. Hence, the top portions of wheat, rye and barley at this early stage, even when cut at 1 in. below the soil surface, contained no nodal tissue capable of regrowth, and all of them died. Somewhat similar results are shown in Table IX in the case of cuttings taken from seedlings three and nine days after emergence. Where material was sectioned at later stages of development on the 12th, 24th and 32nd days after emergence, the results for cereals resemble those for oats sectioned at very early stages.

The vigor of vegetative regrowth in different species is of interest. With both species of oats, rerooting takes place with great readiness and the plants thus produced are as vigorous as those produced from seed, while with other cereals, when regrowth does occur, it is much less vigorous, and has less resistance to unfavorable external conditions.

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SPIROCHAETES OF THE DISTRICT OF MONTREAL AND OF THE PROVINCE OF QUEBEC[1]

By G. Gardner

Abstract

This paper is devoted to a brief discussion of the general characteristics of spirochaetes, including staining and cultures, and to records of observations on spirochaetes in Canada, which are of interest for comparison with those from other parts of the world.

Fresh water from various sources was examined both directly and after collection and cultivation. The results show that while Canadian waters are extremely poor in spirochaetes they do contain various types resembling those recorded from other parts of the world. By maceration of vegetation in sterile tap water a number of spirochaetes were obtained including *S. biflexa*, *S. pseudo-icterohemorragiae*, and in particular a distinct type referred to as the "timothy type", obtained from timothy grass. This spirochaete resembles *S. plicatilis*, but is very much shorter (a maximum of 15 μ as compared with lengths up to 500 μ). The addition of oxazine (1-1000) causes the surface membrane and granulations to become distinct and also reveals the existence of an axial filament.

The intestines of a number of animals, both vertebrate and invertebrate, were examined with negative results, but spirochaetes were obtained by cultivation from the saliva or foam of a cow. A number of rabbits were found to be infected with *S. cuniculi* and to harbor a spirochaete in the caecum. *S. icterohemorragiae* was also detected in the kidneys of wild rats.

Strains of *S. pseudo-icterohemorragiae* (free living) were found to be non-pathogenic. Nevertheless after being cultivated for two or three years, some of these free living strains acquire certain characteristics of the pathogenic type and it is possible that under proper conditions the physiology of certain micro-organisms could be so changed as to render them pathogenic to man.

The influence of cold on spirochaetes was also studied, cultures of *S. pseudo-icterohemorragiae* being kept at a temperature of approximately $-15°$ C. The spirochaetes were found to lose their motility gradually and to break up into granules. Subcultures of these forms were negative. It would seem that the comparative rarity of spirochaetes in Canada may be attributed to the low winter temperatures.

Introduction

The word spirochaete was first introduced into the literature by Ehrenberg (10) in 1838, who at that time was studying two new groups of organisms, Spirillum and Spirochaeta. Owing to a misunderstanding that is difficult to explain, the word and the description of Spirillum have been applied to Spirochaeta, and *vice versa*. Thus, spirochaetes are described as spiral organisms, whereas in reality, they are helicoidal or screw-shaped. Pettit (22) was the first to call attention to this error, the seriousness of which may be judged from the following descriptions of a spirillum and a spirochaete.

Definition of a spirochaete. A unicellular protist, related to the Schizophytes, body flexible, forming a perfect geometric screw, no flagella visible under dark field illumination, movements resembling those of a screw.

Definition of a spirillum. A bacterium, body non-flexible, flagella visible under dark field illumination.

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Contribution from the University of Montreal, Montreal, Canada. This paper was constructed from a thesis presented to the University of Paris in partial fulfilment of the requirements for the degree of Doctor in Natural Sciences.

Spirochaetes

Spirochaetes possess few morphological or physiological characteristics useful for the determination of species. The few useful characteristics are as follows:

Shape. The shape is of morphological significance in that the dimensions of the helix are to a certain extent characteristic for each species.

Helical amplitude. The helical amplitude of the screw is usually difficult to measure as it is of the order of 0.25 μ to 1μ, and in very minute species such as *Sp. biflexa* it may even be impossible to measure it. The helical amplitude varies with different culture media, etc.

Length. The length as well as the helical amplitude varies with different individuals, culture media, etc., and for this reason cannot always be relied upon as an index for the determination of species; for instance, the length of *Sp. eurygyrata* varies between 4μ and 80μ.

Study in vivo. Spirochaeta are best studied *in vivo* under dark field illumination. If the micro-organisms are too active it may be necessary to wait a few minutes to allow the field to clear, but by this means it is always possible to make a distinction between spirochaetes and any other micro-organisms which may appear on the slides. The only objection to the method may be the relative scarcity of spirochaetes, but this difficulty may be overcome by enriching the cultures, thus increasing the relative numbers of spirochaetes.

Experimental

Materials and Methods

The author was obliged to devise special culture methods, since spirochaetes, being neither bacteria nor protozoa, will not develop in the culture media commonly used for such organisms. The technique finally adopted was a modified form of that devised by Dr. M. Zuelzer; spirochaetes being found under dark field illumination, the liquid containing them is centrifuged and the bottom portion transferred to a culture medium.

Some of the principal media used were ordinary hay infusion, 1% peptone in boiled tap water, and solidified agar. The further details of procedure and media will be discussed in connection with each particular case.

Staining

With the exception of a study *in vivo* the best results were obtained by staining with basic aniline dyes, and of these, crystal violet and Ziehl's fuchsin or phenol fuchsin gave the best results. The staining techniques were as follows:

Pulgher technique. Smears are prepared, allowed to stand for two hours and then washed with distilled water. A small quantity of the stain (as per formula) is boiled in a test tube, poured on the slide, allowed to cool, and washed with tap water. Formula of Pulgher stain:— distilled water, 100 cc.; alcoholic tannin, 0.90 cc.; crystal violet, 0.45 gm. After cooling this is mixed in equal parts with a 3% solution of hydrochloric acid in distilled water.

Renaux technique. Smears are fixed with Ruge's formalin (2% formalin in 1% acetic acid) for from two to four minutes. They are then washed with alcohol and covered with a saturated aqueous solution of picric acid. After

standing 10 min. they are washed and stained with Ziehl's fuchsin, made up as follows: fuchsin, 1 gm.; carbolic acid, 5 gm.; absolute alcohol, 10 cc.; distilled water, 100 cc.

The fuchsin is ground with alcohol in a mortar, adding the carbolic acid meanwhile. Two-thirds of the distilled water is then slowly added and the mortar is rinsed with the remaining one-third. The solution is allowed to stand 24 hr., filtered and kept in a well corked bottle.

Silver nitrate method. A silver nitrate method similar to that used in staining nerve tissue is frequently used in the study of spirochaetes, but while the results are striking in appearance the method has the disadvantage that the interior of the helix is completely filled with silver so that it is possible to mistake connective tissue fibres for spirochaetes. On account of the possibility of such an error, the method was not used to any very great extent in the present studies.

Discussion

The spirochaetes studied were those present in water, cow manure, plant infusions, algae and animals. They will be discussed in that order.

Spirochaetes of Water

Spirochaetes have been found in soft waters in many different countries, especially in the following: in Germany by Uhlenhuth (24), Baermann (1), Zuelzer (31, 32) and Hoffmann (15, p. 611); in England by Dobell (9) and Hindle (14); in France by Pettit (22), Etchegoin (11, 12), Cantacuzene (5) and Vinzent (28); and in the United States by Wolbach and Binger (29, 30), and by Noguchi (18, 19, 20, 21).

In Canada the study of Spirochaetes has not attracted much attention in the past though *Treponema pallida* is well known on account of its medical significance, while *Spirochaeta icterohemorragiae* has been observed in Toronto, by Bates (2) and by Cameron and Irwin (4).

In the present study a large number of samples were examined including some from rivers condemned by the Board of Health, but spirochaetes were observed in only a few cases. The spirochaetes found in rivers resembled more or less those found under similar circumstances in Europe, though in many cases considerable differences were observed.

Spirochaetes were present in samples of water from the following places: Campbellton Wharf (sea water), Cross Point (dirty water from a gutter), Matapedia River at Matapedia, Quebec, St. Lawrence River water at Tadousac in Quebec (this water had been lying, for some time, in holes in the wharf), in water from Rivière du Loup at the city of Rivière du Loup in Quebec, and in some water from a tannery. A detailed discussion of these samples follows.

Sea water from Campbellton, N.B. This water, in which there was a small quantity of sand, contained two types of Spirochaetes. These may be described as follows:— Type No. 1: length 5-9 μ, total width 1-1.5 μ, helix with 2-6 loose turns, membranes and granulations in the body visible. Type No. 2: length 10-15 μ, width 0.5 μ, helix with 20-30 very close turns, extremities fine, resistant to 10% saponin, division transversal.

Type No. 1 was similar to *Sp. plicatilis pallida* Zuelzer, but was somewhat thicker and shorter, while Type No. 2 was similar to *Sp. stenostrepa*. A complete study was impossible owing to a scarcity of specimens.

Cross Point. This water contained spirochaetes which may be described as follows: length short, helix with 2-4 turns, measuring 2-3 μ, membrane granular.

Matapedia River. Large spirochaetes showing granulations were found.

St. Lawrence River at Tadousac. This water contained large spirochaetes, length 4-5 μ, membrane granular, similar in appearance to *Sp. plicatilis marina* except for the great difference in length, the latter measuring 125 μ in length. The spirochaete found in the Campbellton water and resembling *Sp. plicatilis pallida* was also present.

Rivière du Loup. Spirochaetes 3-5 μ in length and with transverse division were found.

Tannery water. Samples of water and other liquids used for tanning leather were examined, two types of spirochaetes being observed: Type No. 1: length 2-10 μ, helix with 4-8 turns, membrane visible, granulations absent, individuals instantly dissolved by 30% Javel water. Type No. 2: length 2-4 μ, and 6-9 μ, total width 1 μ, granulations visible. Type No. 2 showed a strong resemblance to *Sp. pseudo buccalis* observed by Zuelzer in Germany.

No spirochaetes were found in tap water in the city of Montreal or in water from two taps in Dr. Pettit's laboratory at the Pasteur Institute in Paris.

Spirochaetes in Cow Manure

From time to time spirochaetes with from two to three turns have been observed in cow manure. In the present study the manure was diluted with boiled water and cultured on agar. A few spirochaetes were obtained in this way but not a sufficient number for a complete study of their characteristics.

No spirochaetes were found in liquid manure though good specimens of *Spirillum* 15 μ in length, 1-2 μ in width and with from three to four undulations were found. These were present in numbers as high as 15-25 in each field.

Spirochaetes of Plant Infusions

A great number of plants were examined in the hope of finding spirochaetes, the best results being obtained with infusions of timothy, *Phleum pratense*. A number of other plants, including *Euphorbia*, potatoes and beans, were examined in an unsuccessful attempt to find spirochaetes. Fresh plants were collected and brought directly to the laboratory where they were placed in sterilized jars containing boiled tap water.

Spirochaetes were usually present after three weeks, and may be described as follows: average length 6-15 μ, width 0.25-0.50 μ, helix with 2-8 turns (pH; 6.6-6.8). These spirochaetes represent a new species which will be discussed later. They were found in some infusions for over three months and may be temporarily referred to as *Spirochaeta of timothy*.

Other micro-organisms recognized in these infusions were: *Bodos* (sp?) which appear a few days before the spirochaetes and remain two or three weeks longer; *Sp. biflexa*; and a large bacterium with long flagella which, although observed by Noguchi (21), has not yet been named.

It is of interest to compare the morphological characteristics of *Sp. of timothy* with those of various other common spirochaetes. This comparison is made in Table I.

TABLE I

A COMPARISON OF THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF *Sp. of timothy* AND THOSE OF VARIOUS OTHER SPIROCHAETES

Name	Length in microns	Width in microns	Stability of shape with various media	Stability of shape when stained	Remarks
Sp. of timothy	6–15	.25–.50	Retains shape	More change than in case of *pseudo-pallida* or *recurrentis*	Similar in appearance to *Sp. plicatilis*, but shorter, aerobic, retained by Chamberland L3 filter
Sp. graminea	100	2.5	Shape irregular	Shape irregular	Similar in appearance to *Sp. stenostrepa*, less refringent
Sp. plicatilis	500	.25–.50	Changes shape slightly	Axial filament strongly stained	See remarks on *Sp. of timothy*
Sp. recurrentis	6–15	Greater than *Sp. timothy*	Changes shape	Less change than in case of *Sp. of timothy*	
Sp. calligyrum	Shorter than *Sp. timothy*	Thinner than *Sp. timothy*			Anaerobic, screw heavier than *Sp. pallida*
Sp. biflexa	5–7	0.2	Retains shape	Slight changes, ends remain curved	Number of turns in helix similar to *Sp. of timothy*

From Table I it is apparent that *Sp. of timothy* is not identical with any of the other spirochaetes in the table, and in view of other careful comparisons yielding similar results the author believes it to be a new species.

It appears to be fairly common, since it can be obtained from either green or dried timothy, or even from material that has been in the field during the winter. Growth can be stimulated by the addition of 1% peptone to the infusions. It is also possible to obtain it from hay infusions kept at pH 6.6 for a month. An unsuccessful attempt was made to secure pure cultures in Montreal and at the Pasteur Institute in Paris.

A few additional notes on the morphology and physiology of *Sp. of timothy* follow.

Morphology. *Sp. of timothy* sometimes contains granulations, and after staining, the body flattens slightly, though the screw remains apparent. The membrane covering the contours is clearly visible and represents about one-fifth of the total thickness of the organism.

Physiology. *Sp. of timothy* shows interesting reactions to various chemical substances. It is killed after a few minutes contact with bile salts (10%), and is dissolved after half an hour's contact with the same solution. It is rapidly dissolved by commercial Javel water diluted to one-third with ordinary water. Saponin (10%) produces granulations in the body.

A few experiments were also made with oxazine. On placing the organisms in an aqueous solution of oxazine (1/1000) they were not stained but the membrane and granulations became more distinct while the axial filament in particular became very distinct. After some time death occurred, the remaining portions, *i.e.*, the axial filaments, adhering to each other in chains, forming figures-of-eight.

The organism normally moves with a screw-like motion but on reaching the granular stage the movement becomes slower and finally stops.

It was thought at the beginning of the present study that the pH would vary considerably in the cultures, but the contrary proved to be the case, and after numerous observations it was discovered that in the case of any one culture, a decrease in the number of spirochaetes was due to an exhaustion of organic material rather than to a change in pH.

Bodos (sp ?) were sometimes very numerous in the author's cultures, and proved to be a sure indication of the future presence of spirochaetes. There is possibly a symbiotic relationship between the two.

Spirochaeta biflexa

It has been mentioned that *Sp. biflexa* sometimes occurred in the author's cultures, particularly in infusions of timothy. This was however only an accidental host.

Sp. biflexa has been found in the water of Jamaica Pond, in Boston, by Wolbach and Binger (30) and has been studied in Germany by Zuelzer (31, 32) and in America by Dimitroff (6, 7, 8) and by Wolbach and Binger (29, 30).

The correct name of this spirochaete has long been a controversial matter; we shall therefore use the name first applied to it. Zuelzer considered that the species *Sp. pseudo-icterogenes* discovered by her was identical with *Sp. biflexa*, but this identity is doubtful since the former is sometimes pathogenic for the guinea pig, the latter never.

Sp. biflexa seems to be world-wide in distribution though individuals are always scarce.

The organism as found in the author's cultures differed slightly from that found by Wolbach and Binger as is shown by Table II.

Sp. pseudo-icterohemorragiae

This organism was found in some of the author's cultures but individuals were never numerous. They appeared to be very similar to the European species, their description being as follows: length 10-15 μ, helix with 16-22 turns, active individuals with very delicate coils.

The position and importance of this organism in nature will be discussed later.

TABLE II

A COMPARISON OF THE MORPHOLOGICAL CHARACTERISTICS OF *Sp. biflexa* AS DETERMINED BY WOLBACH AND BINGER AND BY GARDNER

Authority	Length in microns	Width in microns	Helical amplitude	Number of turns in helix
Wolbach and Binger	5–7	0.2	0.2–0.25	22–32
Gardner	10–12	0.3	0.2	20–40

Spirochaetes in Algal Infusions

Infusions were also made of blue and green algae taken from a spring. Boiled tap water was added to the culture which shortly became filled with bacteria, spirilla and diatoms of various species. Spirochaetes appeared in 10 days. The species present were: *Sp. of timothy*, *Sp. biflexa* and *Sp. plicatilis*.

Spirochaetes of Animals

Considerable work was done on animals in an attempt to discover spirochaetes but it was found that only a small proportion of those examined harbored these organisms.

Cow. The saliva or foam from the mouths of several cows was examined as long as possible after feeding. It was found to be difficult to obtain these materials between periods of rumination. On examination under dark field illumination no spirochaetes were seen, but were obtained in from two to four weeks on placing the saliva in boiled tap water with peptone, or upon hay agar. They were of course mixed with great numbers of bacteria.

These spirochaetes may be described as follows: length 3-10 μ, width about 1 μ, helix with 2-6 well developed turns, membrane visible, body containing granulations. Dr. Noguchi (21), in the United States found a spirochaete in the gastric mucosa of an ox; he states, "I detected a minute Leptospira in the gastric mucosa of the ox (*Leptospira bovis* n.sp.)." This description is so brief that it is not possible to make any comparison between the two organisms.

It was thought at first that these spirochaetes might resemble those from timothy, since in the province of Quebec, cows are fed, to some extent, on this plant. Examination showed, however, that the two differed greatly in both length and width.

Rabbit. In the province of Quebec as well as in other countries rabbits are parasitized by a spirochaete, *Sp. cuniculi*, and this phenomenon has been the subject of much study by many investigators. The species was observed by the author on many occasions and found to have the following characteristics: length 1-16 μ, width 0.25 μ, helix with 6-9 or 16 turns.

This species is so much like *Treponema pallida* that many workers are unable to distinguish between the two without inoculation.

A second species of spirochaete, bearing a close resemblance to those found by Sanarelli (23) in the caeca of Italian rabbits was found by the writer in the caeca of Canadian rabbits and appears to have been observed only by Sanarelli and the author.

The method used in this case was as follows: the contents of the caecum were placed in boiled tap water with added peptone and after the appearance of spirochaetes some of the liquid was transferred to a tube with an agar slant, adding enough liquid to cover the slant completely. The organisms usually grew more abundantly in the upper part of the liquid.

The only differences between the spirochaete found by Sanarelli and that found by the author is that the latter is slightly thicker, and that the extremities are blunt instead of pointed. It bears little resemblance to *Sp. eurygyrata* of the human intestine, this species varying in length from 4-56 μ, while that obtained from rabbits was relatively constant in length.

Rat. Spirochaetes very similar in appearance to those found in rabbits were also found in the caecum of a rat. The kidneys of the same rat contained *Sp. icterohemorragiae* (see next paragraph).

The Problem of Sp. icterohemorragiae in Nature

The work of Inada and his coworkers (16) has shown a relationship between human spirochaetosis and the presence of *Sp. icterohemorragiae* in the soil. This observation was the beginning of research in many different parts of the world. Uhlenhuth and Fromme (25) discovered in stagnant water near Berlin a spirochaete having all the characteristics of *Sp. icterohemorragiae* and named it *Sp. icterogenes* Uhlenhuth and Fromme. It was renamed *Sp. pseudo-icterogenes* Uhlenhuth and Zuelzer, and later Dr. Zuelzer believed it to be identical with *Sp. biflexa* Wolbach and Binger. Later, Noguchi (21) searched for *Sp. pseudo-icterogenes* in various regions of the United States and found "organisms indistinguishable from *Leptospira icterohemorragiae.*"

Professor Inada has given to the micro-organism of spirochaetal jaundice the name of *Sp. icterohemorragiae* and the author believes with Dr. Pettit that the correct name for free living spirochaetes of the group bearing such a strong resemblance to *Sp. icterohemorragiae* should be *Sp. pseudo-icterohemorragiae.*

Sp. icterohemorragiae may be described as follows: average length 9 μ, extreme lengths 3-40 μ, helix with about 20 turns, helical amplitude about 0.5 μ, division transverse, an ecto-parasite of cells, very pathogenic for the guinea pig, less so for man, the causal agent of a jaundice with karyokinesis, and hematophagia, may be cultivated on relatively non-nutritious media, physiological serum, rabbit serum, etc., optimum temperature 29° to 30° C., optimum pH 7.7, does not resist heat, desiccation, traces of acids, bile salts *in vitro*, and is not attacked by 10% saponin. It is agglutinated and lysed by antispirochaetic serum, and by the serum of patients that have recovered from spirochaetal jaundice.

This description is sufficient to identify a micro-organism living in the tissue of a mammal, but it is another matter to identify a free living spirochaete of the groups *icterohemorragiae* or *pseudo-icterohemorragiae* as all the species are so similar in appearance. Morphological characters are not sufficient to distinguish *Sp. icterohemorragiae* from *Sp. pseudo-icterohemorragiae*, and as for pathogenicity, spirochaetes of neither group are necessarily pathogenic for the guinea pig when taken directly from the soil or from water.

Buchanan (3, p. 48) did succeed in producing spirochaetal jaundice in two guinea pigs by inoculating them with mud, but the case is unique, and even Buchanan himself was not able to repeat the result. The author attempted to do so at the Pasteur Institute, but failed.

It appears then that the pathogenicity of free living *Sp. icterohemorragiae* cannot be determined positively. Serological characters are not of much use, the antispirochaetal serum of Pettit and the serum of patients convalescent after spirochaetal jaundice having no effect upon free living *Sp. pseudo-icterohemorragiae.*

The author has observed numerous strains of *Sp. pseudo-icterohemorragiae,* none of which were pathogenic for the guinea pig, the serological reactions being uncertain. It was then thought that possibly these characteristics are acquired only after several passages, through the bodies of animals or cultures, the hypothesis being based on the following experiment with a *Sp. pseudo-icterohemorragiae,* Vinzent's strain.

Vinzent (28) in 1926 isolated a strain of *Sp. pseudo-icterohemorragiae* from sewer water and having no time to continue his work with that strain, left it at the Pasteur Institute in Dr. Pettit's laboratory. The object of the author's experiment was not to verify the experiments of Vinzent and Michailoff, a later worker with that strain, but rather to determine if, after a year of passages, certain properties of the micro-organism had not been modified.

Comparative tests before and after the series of passages were conducted with *Sp. icterohemorragiae* and *Sp. pseudo-icterohemorragiae* in connection with Pfeiffer's phenomenon and agglutination, the results being as follows: In the case of Pfeiffer's phenomenon, the results were about the same for both species but the reaction was more intense in the case of *Sp. icterohemorragiae.* As regards agglutination both organisms are in general agglutinated by convalescent's serum but *Sp. pseudo-icterohemorragiae* is agglutinated only by sera that strongly agglutinate *Sp. icterohemorragiae.*

In 1927 Michailoff (17) wrote: "Vinzent's strain was not agglutinated by anti-icterohemorragiae serum at 1/100, 1/50 and even 1/10." The main fact of these experiments is that previous to the series of transfers Vinzent's strain was not agglutinated but after the series it was so affected. The hypothesis may then be proposed that free living forms may have physiological properties quite different from those of individuals of the same species taken from the bodies of animals. It is possible that under certain conditions the physiology of certain micro-organisms could be so changed as to render them pathogenic to man.

Effect of Low Temperatures on Spirochaetes

Infusions and cultures of various spirochaetes (those found in timothy, liquid manure, and in the caeca of rats and rabbits) were exposed to low temperatures and it was observed that the individuals became shorter, division ceased or became less intense, the helices became less delicate, narrower and more compact, and that the production of coccoid bodies was hastened.

The organisms did not seem much affected by exposure to ordinary low temperatures of only a few hours duration but were killed by a prolonged exposure

to a temperature of −2° C. They were not affected by exposure to a temperature as low as −20° C., provided that the exposure was not greater than 30-60 min.

In these experiments 20 tubes were inoculated with a pure culture of *Sp. pseudo-icterohemorragiae* and kept for four days at laboratory temperature. The cultures developing well, 16 tubes were placed in a refrigerator at a temperature of −15° C., the other four remaining at laboratory temperature.

After 27 days, the tubes kept in the refrigerator showed only small spots or dots under dark field illumination, while in the case of those kept at laboratory temperature, it was possible to obtain spirochaetes by inoculating other tubes from them. It proved impossible to obtain spirochaetes by inoculation of other tubes with material from the tubes kept in the refrigerator.

The fact that the tubes kept in the refrigerator contained only "coccoid bodies" disproves the hypothesis that these bodies are part of any mechanism of division. They represent rather disintegration products.

Gieszczykiewicz (13), working in Dr. Pettit's laboratory obtained much information with regard to these coccoid bodies. The author's results confirm the findings of Gieszczykiewicz.

Conclusions

An attempt has been made to determine the representative spirochaetes of Canada, a certain number of free living and a few parasitic or commensal species being found. It seems that cold is responsible for the scarcity of spirochaetes in the Montreal district.

Types of spirochaetes very similar to those found in Europe were observed, the small differences being due, perhaps, to the differences in local conditions. Other types were noticed, but owing to the difficulties of nomenclature in spirochaetes, the author prefers not to name them.

The question of the screw shape of spirochaetes was considered, and the presence of the axial filament in living spirochaetes was demonstrated by the use of oxazine.

The presence of *Sp. icterohemorragiae* in Montreal rats and of *Sp. pseudo-icterohemorragiae* in a free living condition was demonstrated. It was also observed that free living strains of the latter have physiological characteristics differing from those of individuals obtained from the bodies of mammals, but that after a long series (2-3 years) of passages through the bodies of mammals or cultures, the physiological characteristics of the free living type approached those of the parasitic type. It was then suggested that certain normally harmless micro-organisms might become pathogenic by reason of serial transfer in the above manner.

Finally the effect of low temperatures on spirochaetes was investigated and a series of observations made on the effect of an exposure to −15° C. This exposure resulted in certain morphological changes culminating in death.

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REVIEWS AND NOTES

LIST OF FUNGI INFECTING MAN IN MANITOBA[1]

By A. M. Davidson[2] and P. H. Gregory[3]

In the course of investigations in medical mycology now in progress in Winnipeg under a grant from the Banting Research Foundation, Toronto, records have been kept of cases of fungus infection treated at the General Hospital and at the Children's Hospital, Winnipeg. The list of species isolated is published with the belief that it will prove of interest for comparison with similar lists compiled by workers in other localities.

The present account is preliminary in scope. The period covered by the observations extended from September 1930 to June 1932. The nomenclature used by Sabouraud has been adhered to as being that most widely known. *Microsporon felineum* Fox and Blaxall, is provisionally taken as including *M. lanosum* Sabouraud.

The number of patients from whom each species has been isolated is given in the right hand column of Table I. The fungi were determined in culture on maltose "Proof medium", in the preparation of which were used maltose "bruit de Chanot" and peptone "Chassaing" as postulated by Sabouraud. In addition, Dr. E. Muskatblit of New York has kindly identified a number of the cultures.

In recording a somewhat smaller proportion of fungal lesions of the glabrous skin (ringworm of the feet, etc.) than probably occurs, Table I does not present quite a true picture of the incidence of the various clinical types of skin infection in Winnipeg. The cause of this is that up to the present time work has been concentrated largely upon affections of the hairy skin. On this account some cases of epidermophytosis may possibly have escaped investigation in the laboratory.

From the data given in Table I, several interesting facts emerge:

(a) There is a comparative paucity of species of dermatophytes so far recorded in Manitoba. Sabouraud (3, p. 582) isolated 28 species from 500 cases of dermatomycosis in Paris. Burgess (1) in Montreal isolated 23 species from 99 cases.

(b) A striking divergence from the records of Sabouraud (3, p. 582) is the relative infrequency in Winnipeg of cases of tinea capitis caused by species of *Trichophyton*. This is emphasized in Table II, in which the data of Burgess for Montreal and of Catanei for Oran, Algeria, are included for comparison.

(c) *Trichophyton crateriforme* Sabouraud (*T. tonsurans* Malmsten), responsible for 112 out of Sabouraud's 377 cases of tinea capitis, has not been isolated at Winnipeg.

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TABLE I

LIST OF FUNGI INFECTING MAN IN MANITOBA 1930–1932

	Origin	Number of isolations
Microsporon audouini Gruby		38
tinea capitis	36	
tinea corporis	2	
Microsporon felineum Fox and Blaxall		18
tinea capitis	12	
tinea corporis	8	
infection from cat suspected	9	
Trichophyton violaceum Sabouraud		4
tinea capitis	4	
Trichophyton (gypseum) spp. Sabouraud		4
tinea barbae	2	
tinea corporis	1	
tinea pedis	1	
Trichophyton (faviforme) album Sabouraud		4
tinea capitis, kerion	1	
tinea corporis	1	
tinea barbae	2	
tinea ciliaris	1	
Epidermophyton inguinale Sabouraud		2
tinea cruris	2	
tinea pedis	1	
Achorion schoenleini (Lebert) Remak.		4
favus	4	
**Penicillium (Scopulariopsis) brevicaule*		1
tinea corporis	1	
†*Monilia (Candida)* spp. (*M. albicans ?*)		10
moniliasis of mouth	3	
erosio interdigitalis blastomycetica	8	
Total		85

**Pathogenicity regarded as doubtful.* †*Pathogenicity regarded as doubtful in certain cases.*

TABLE II

DISTRIBUTION OF SPECIES OF DERMATOPHYTES CAUSING TINEA CAPITIS IN PARIS, MONTREAL, ALGERIA AND WINNIPEG

Organisms causing tinea capitis	Number of cases			
	Paris 1907–1909	Montreal 1925	Algeria 1931	Winnipeg 1930–1932
Microsporon species	159	41	1	48
Trichophyton species	218	13	28	5

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